The synthesis of potentially sweet dihydrochalcone glycosides.

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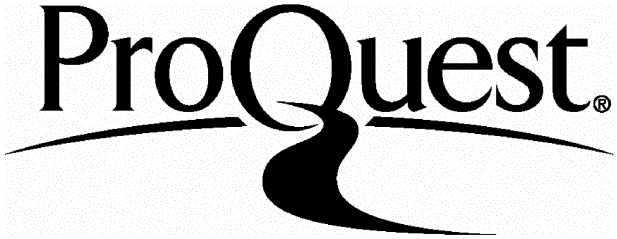
THE SYNTHESIS OF POTENTIALLY SWEET DIHYDROCHALCONE GLYCOSIDES

submitted by CHRISTOPHER MICHAEL NOBLE
for the degree of Doctor of Philosophy
of the University of Bath.

1974



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C O N T E N T S

SUMMARY

Several dihydrochalcone glucosides have been synthesised and their potential value as possible sweetening agents have been assessed.

Some of these dihydrochalcone glucosides were isolated as gums. These were the glucosides of 4'-hydroxydihydrochalcone; 4,4'-dihydroxydihydrochalcone; 3,4'-dihydroxy-4-methoxydihydrochalcone; 4'-hydroxy-3,4-dimethoxydihydrochalcone and 4'-hydroxy-2-methoxydihydrochalcone. Two more of these compounds were obtained as solids, however. These were: 4,4'-dihydroxydihydrochalcone as a pale yellow, waxy solid and 4'-hydroxy-3,4-methylenedioxydihydrochalcone as a white, crystalline solid. All these dihydrochalcone glucosides were found to be non-sweet.

The dihydrochalcone aglycone 3,4'-dihydroxy-4-methoxydihydrochalcone produced an enhanced sweetness sensation when added to 5% w/v solutions of either glucose, sorbitol or mannitol.

Naringin dihydrochalcone and Neohesperidin dihydrochalcone were prepared and their sweetness characteristics were assessed in Quosh and Chekwate Orange Drinks. These sweeteners produced a delayed sweetness effect, a gasp effect and their sweetnesses were of a lingering, cloying nature. In Quosh Orange Drink the sweetness equivalence of neohesperidin dihydrochalcone was found to be 3.8 X saccharin at the concentration used. The corresponding figure for naringin dihydrochalcone was 0.19 X saccharin. In Chekwate Orange Drink the sweetness equivalences for neohesperidin and naringin dihydrochalcones were 6.4 and 0.21 X saccharin, respectively.

It is suggested that the glucose and rhamnose constituents of the neohesperidose moiety in neohesperidin dihydrochalcone adopt the CI conformation as it interacts with the human taste-bud receptor. This conformation would allow two pairs of OH groups to adopt the gauche conformation so that the inter-hydroxyl distance is ca. 3A. If the IC conformation were adopted, only one hydroxyl pair would adopt the gauche conformation with attendant diminution in sweetness intensity.

Attempts to prepare dihydrochalcone disaccharides from simple starting materials were unsuccessful. The compounds investigated were: 4-hydroxyacetophenone-4-β-neohesperidoside; 4-hydroxyacetophenone-4-β-sophoroside; 4-hydroxyacetophenone-4-β-laminaribioside and 4-hydroxyacetophenone-4-β-maltoside.

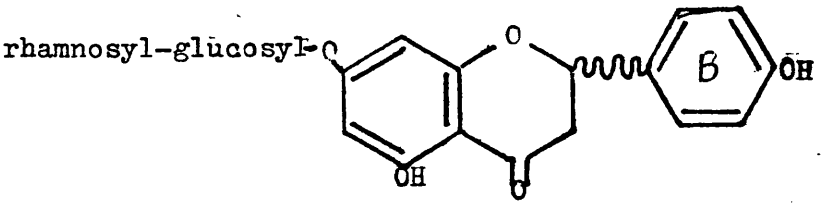
PART A

DIHYDROCHALCONES

A REVIEW

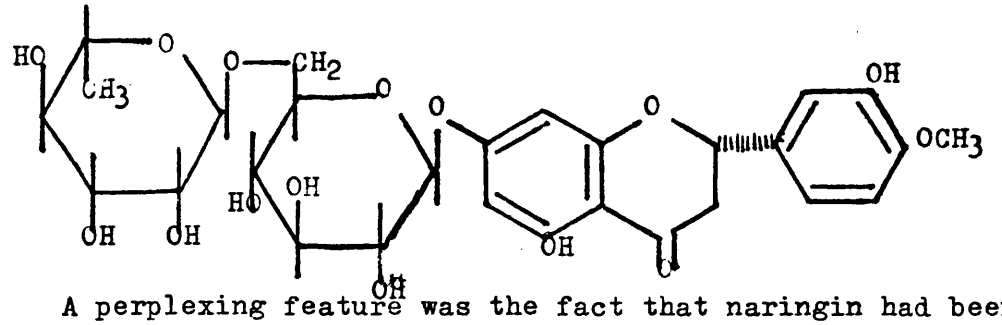
I.Introduction.The Discovery of Dihydrochalcone Sweeteners.

In 1958,R.M.Horowitz and B.Gentili of the U.S.Department of Agriculture,Fruit and Vegetable Chemistry Laboratory,Pasadena, California,commenced a study on the relationship between the structure and taste of the phenolic glycosides occurring in citrus fruits.[1] They were concerned chiefly with naringin,the major bitter principle of grapefruit,whose structure at the time was known to be a flavanone rhamnosyl-β-glucoside but the point of attachment of the rhamnose to the glucose was unknown.(I).



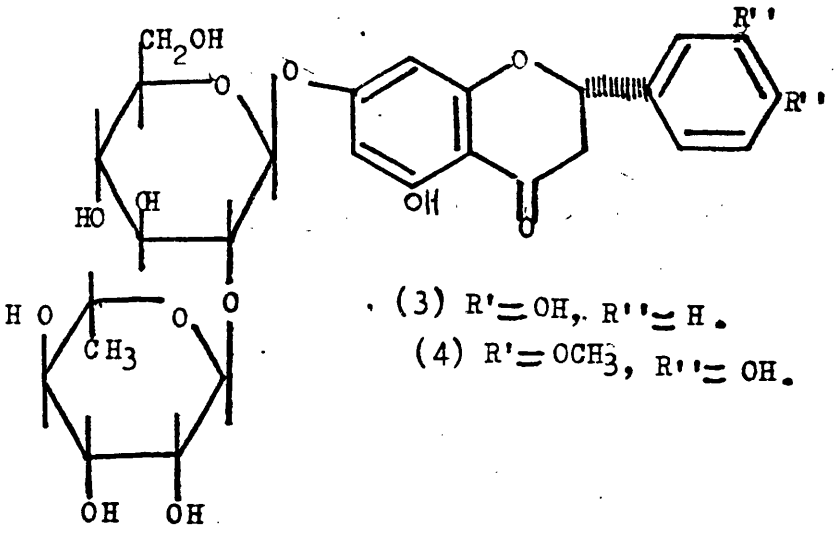
(I)

The structure of a related citrus glycoside,hesperidin was known in detail as being the 7-β-rutinoside of the flavanone aglycone 2(S)-hesperidin.(2).



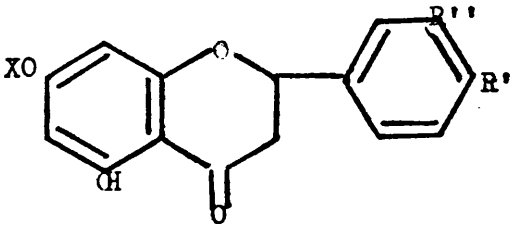
(2)

A perplexing feature was the fact that naringin had been described in the literature as a rutinoside differing from hesperidin only in the substitution pattern in the "B" ring,yet hesperidin itself is tasteless.Horowitz and Gentili proved that the dissaccharide component in naringin is 2-O-α-L-rhamnopyranosyl-β-D-glucopyranose by methylation of the naringin and identification of the methylated carbohydrate products.These authors also showed that neohesperidin contains the same dissaccharide,neohesperidose-this compound having been named before its structure was known. The structures of both naringin(3) and neohesperidin(4) are shown below.The results implied that the bitterness of naringin versus the tastelessness of hesperdin is due to the difference in the point of attachment of the rhamnose to the glucose.This belief was reinforced when it was found that neohesperidin was bitter and of the flavanones shown in Table I those containing the rutinose moiety are tasteless and those containing the neohesperidose residue are bitter.



(3) R'=OH, R''=H.
(4) R'=OCH_3, R''=OH.

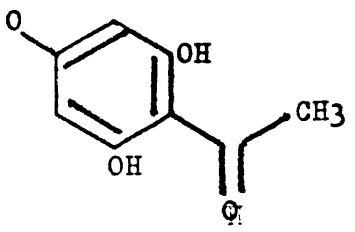
TABLE I. Flavanone Glycosides of Citrus Fruits.



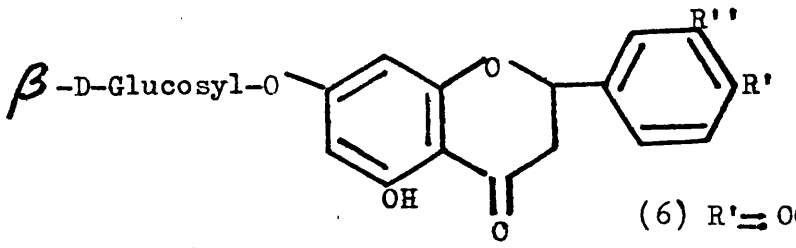
X Rutinosyl	X Neohesperidosyl	R'	R"
Hesperidin	Neohesperidin	OCH_3	OH
Naringenin rutinoside	Naringin	OH	H
Isosakuranetin rutinoside	Poncirin	OCH_3	H
Eriocitrin	Neoeriocitrin	OH	OH

Having established the relationship between dissacharide structure and bitterness for this group of compounds Horowitz and Gentili then tried to determine other structural requirements for taste. They found that the presence of rhamnose at the 2-position of glucose is not essential for bitterness to result though in some instances it may enhance the bitter or sweet taste by an order of magnitude or more. In contrast, rhamnose at the 6-position of glucose seems to abolish the taste effect, regardless of the nature of the aglycone. That the "B" ring is not required for bitterness is illustrated by compound (5). Examples of glucosides which are bitter are compounds (6) and (7).

Neohesperidosyl-

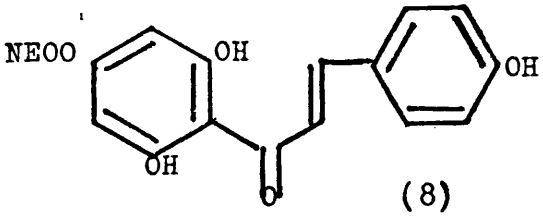


(5)

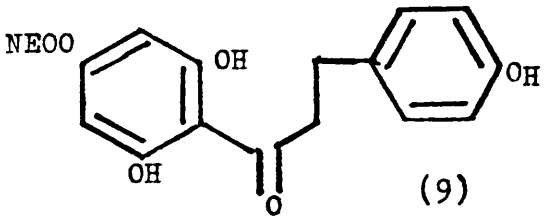


(6) R'= OCH_3, R''=OH;
(7) R'=OH, R''=H.

One of the transformations which was tried was the conversion of naringin(3) to its chalcone(8) followed by catalytic hydrogenation to the dihydrochalcone(9). (see Section 4)

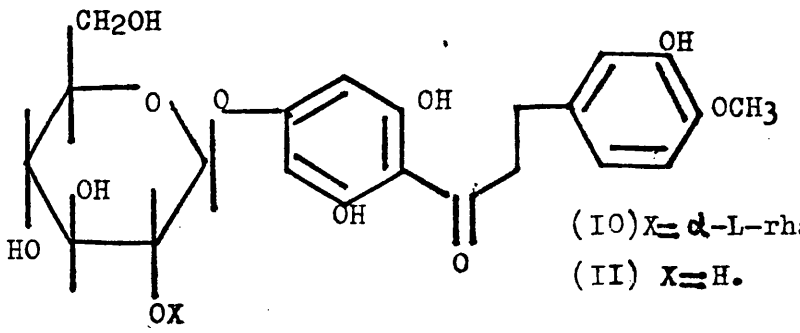


(8)



(9)

Surprisingly, both compounds(8) and (9) were intensely sweet and this result prompted the investigation of the taste properties of the dihydrochalcones of the flavanones listed in Table I. Only one of these, neohesperidin, yielded a sweet dihydrochalcone whereas poncirin dihydrochalcone was mainly bitter and neoeriocitrin dihydrochalcone was, at most, slightly sweet. The dihydrochalcones which are of greatest interest as potential sweeteners are naringin dihydrochalcone(9), neohesperidin dihydrochalcone(10) and hesperetin dihydrochalcone 4'-β-D glucopyranoside(11) and preparations of these compounds are described in Section 4).



(IO) X= α-L-rhamnopyranosyl.
(II) X=H.

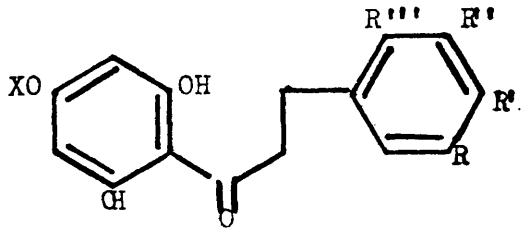
Naringin is readily available from grapefruit and neohesperidin can be obtained from the Seville orange (Citrus aurantium). The quantity of this fruit available is relatively small, however, and it would be necessary to prepare neohesperidin dihydrochalcone from naringin by chemical modification (see Section 4) if this material is required in large quantities. Of the three compounds described neohesperidin dihydrochalcone is outstanding for its high level of sweetness and solubility. Although each compound has somewhat different taste characteristics, in general they exhibit a sweetness which is slow in its onset and of long duration. There is no bitter aftertaste but there is a sensation vaguely reminiscent of licorice or menthol.

Dihydrochalcones are obviously of great interest to those manufacturers of products who require low calorific sweeteners and this interest has been intensified since the ban on cyclamate sweeteners has been enforced in the United Kingdom, the U.S.A. and elsewhere. Additionally the toxicology of saccharin is being actively investigated in several laboratories following the evidence of carcigenicity which was found in experimental animals at the Wisconsin Alumni Research Foundation in the U.S.A. All this uncertainty has hastened the search for new alternative sweeteners. Thus, dihydrochalcones are of interest because two of them (naringin and neohesperidin dihydrochalcones) can be prepared by a simple two-step process (i.e. chalcone formation followed by hydrogenation) from naturally occurring materials. Paradoxically the bitter flavanones naringin and neohesperidin can be converted to intensely sweet compounds. The fact that naturally occurring materials would be used and that the naringin and neohesperidin is subjected only to ring-scission followed by hydrogenation indicates that public acceptance would be forthcoming for a process which is not much more complicated than the hydrogenation of vegetable oils to make margarine. Of course, the belief that natural materials are likely to be non-toxic is erroneous and toxicological work is necessary. A major study with rats has been completed, in fact, and has not revealed any undesirable side effects. (see Section 7).

The development of dihydrochalcones has reached the pilot stage at the U.S. Department of Agriculture's Western Regional Research Laboratory in Albany California.[2,3] Nutrilite Products, Inc. of Buena Park, California and California Aromatics and Flavors Inc. California have also manufactured pilot scale quantities of dihydrochalcone

Krbechek and Inglett[4] extended the work of Horowitz and Gentili by preparing (see Section 4) analogues of naringin dihydrochalcone in which the 'B' ring was replaced by others with different substituents. These dihydrochalcones, those prepared by Horowitz and Gentili and others are summarised in Table 2 together with their sweetnesses, if known.

TABLE 2 A summary of known dihydrochalcones and their sweetnesses.



No.	Name	X	R	R'	R"	R"'	Sweetness (sucrose 1)	Ref
12		NEO	H	H	H	H	0	
13		NEO	H	H	H	OH	sl.bitter	4
14		NEO	H	H	OH	H	110	4
9	Naringin DHC	NEO	H	OH	H	H	110	4
15	Neoeriocitrin DHC	NEO	H	OH	OH	H	sl.sweet	1
16	Poncirin DHC	NEO	H	OCH_3	H	H	bitter/sweet	1
10	Neohesperidin DHC	NEO	H	OCH_3	OH	H	950	4
17		NEO	H	OEt	OH	H	1100	4
18		NEO	H	OPr^n	OH	H	2000	4
19		NEO	OH	OH	OH	H	0	1
20		NEO	H	OPr^i	OH	H	sweet	1
21		NEO	H	H	OCH_3	OH	sweet	1
22		NEO	H	OH	OEt	H	0	1
23		NEO	OH	OCH_3	OH	H	0	1
24		NEO	H	OCH_3	OH	OCH_3	0	1
25		NEO	H	OCH_3	OCH_3	H	bitter/sweet	1
26		NEO	H	OH	OCH_3	H	0	1
27		NEO	H	OCH_3	CH_3	H	0	1
28	Hesperidin DHC	RUT	H	OCH_3	OH	H	0	1
29	Naringenin DHC rutinoside	RUT	H	OH	H	H	0	1
11	Hesperetin DHC glucoside	GLU	H	OCH_3	OH	H	sweet	1
195	Prunin DHC	GLU	H	OH	H	H	sweet	1
31	Phloridzin	2'-OH GLU	H	OH	H	H	0	1
32	Glycyphyllin	2'-OH RHA	H	H	H	H	bitter/sweet	1

Krbechek and Inglett have also published their findings on the sensory and stability characteristics of dihydrochalcone sweeteners. They report that neohesperidin dihydrochalcone gave a good quality taste sensation when 25% neohesperidin D H C was combined with 64% saccharin and 11% cyclamate. The lingering and cooling characteristics of the neohesperidin dihydrochalcone preclude its use when other sweeteners are absent and it must be used in combination with other sweeteners. It was found, for example, that a mixture of neohesperidin dihydrochalcone and cyclamate in a 50:50 ratio on a sucrose equivalence basis imparted a more quickly perceived, pleasanter sweetness to gelatin than a 90:10 mixture or neohesperidin dihydrochalcone alone.

The sweetness of neohesperidin dihydrochalcone appeared to be diminished by a decrease in P_H in aqueous solutions. A solution containing 0.01% neohesperidin dihydrochalcone (approximately equivalent to 10% sucrose), adjusted to P_H. 2.5. with phosphoric acid was less sweet than a solution without phosphoric acid. A 10% solution of sucrose at P_H. 2.5. had the same sweetness as a solution of the sugar alone. Solutions of neohesperidin dihydrochalcone which were adjusted to P_H values of 6.2, 4.5, 3.5, and 2.5 with citric acid showed diminishing sweetness as the P_H decreased. Solutions of the sweetener adjusted to an alkaline P_H of 7.6 and 8.8 had a slightly diminished sweetness with a softening of the harsh note. Lemon flavoured products had a greater apparent tartness when they contained neohesperidin dihydrochalcone which suggested a possible synergistic action.

Table 3 Lemonade Taste Qualities with various sweeteners.

	Lemonade, Taste Quality	
	Sweetness.	Tartness
Sucrose	Typical sweet-tart	Mod. lemon tart
Saccharin	Same as sucrose	Less tart than sucrose
Cyclamate	Same as sucrose	Less tart than sucrose
Neohesperidin DHC	Delayed, less than sucrose	More tart, harsh, acidic.

Combinations of neohesperidin dihydrochalcone with sodium chloride, sucrose, monosodium glutamate, citric acid and dilute lemon juice were also tried in order to determine possible synergistic effects. At near threshold levels of both the sweetener and the test compound there appeared to be a slight effect on salt and acid perception. There was no additive or synergistic effect with sucrose. Monosodium glutamate seemed to enhance the non-sweet quality of the neohesperidin dihydrochalcone. With higher levels of salt or acid and near threshold levels of neohesperidin dihydrochalcone no effect was noticeable.

Krbechek and Inglett also determined the stability of neohesperidin dihydrochalcone to acidic hydrolysis by determination of the free sugar (rhamnose and glucose) present in aqueous solutions of various acids at various PH levels and temperatures. There was little difference between the extent of hydrolysis produced by the various acids. At room temperature the glycosidic bonds of neohesperidin dihydrochalcone in aqueous solution are resistant to hydrolysis by various acids above P_H. 2. For example, at 50°c and at P_H 2.5 a solution of neohesperidin dihydrochalcone was resistant to hydrolysis until after fourteen days some free sugar was detected. At 50°c and at P_H 1.5. free sugar was detected after seven days. At 75°c and at P_H 2.5 free sugar was detected after one hour.

It is worth drawing attention to several statements made by these authors. Firstly, " the lingering quality and cooling effect are also potent. Thus in most products the use of neohesperidin dihydrochalcone to supply all the desired sweetness presents some problems".

Secondly, " Various casual observations indicated that the sweeteners may not be entirely stable under oxidative conditions or in combinations with certain compounds....". These authors also note that the n-propyl analogue has 'less apparent aftertaste' compared with neohesperidin dihydrochalcone.

The likely maximum availability of dihydrochalcones from grapefruit naringin, their cost and the likely demand has been investigated by the author.[6] The Commonwealth Secretariat published their latest edition of 'Fruit - A Review in 1970[7] and the most up-to-date figures for grapefruit production are cited for the year 1968. Thus, in 1968 the total production of grapefruit in the principle producing countries was 2,742,000 tonnes.

Of this total 2,112,000 tonnes (77%) were produced in the U.S.A. and 259,000 tonnes (9.4%) were produced in Israel. Also, the mean percentage of grapefruit processed in the U.S.A. in the years 1966- 67, 1967 - 68 and 1968 - 69 was 55%.

Therefore, of the 1968 U.S.A. grapefruit crop of 2,112,000 tonnes the amount processed was 1,162,000 tonnes if the assumption is made that the proportion processed was 55% of the total crop.

Given that a grapefruit consists of 30% w/w peel[8] then the maximum amount of peel theoretically obtainable from the 1968, U.S.A. processed crop was 349,000 tonnes.

Because the peel contains ca. 1.7% w/w naringin[8] the amount of naringin obtainable from the peel was 5933 tonnes.

Extrapolating the U.S.A. figures to the world crop of 2,742,000 tonnes the total amount of naringin available was ca. 7700 tonnes.

This quantity of naringin would yield ca. 3000 tonnes of neohesperidin dihydrochalcone given a yield of 48%. This quantity of dihydrochalcone is equivalent to ca. 6000 tonnes of saccharin. At the moment, the approximate world-wide yearly production of saccharin is 10,000 tonnes. It is possible to calculate an approximate costing for the production of neohesperidin dihydrochalcone.

The cost of raw materials required to process 1 kg. naringin are as follows:

	£ - p
1 kg naringin	8 - 00
260 g isovanillin	2 - 50
50 g catalyst	1 - 70
4 kg potassium hydroxide	3 - 00
ca.	£14 - 00
Assume a manufacturing cost of ca.	£14 - 00/kg.
Total cost ca.	£28 - 00 kg.

Therefore the cost per kilogram of neohesperidin dihydrochalcone is estimated at £70 - 00. The cost comparisons between neohesperidin dihydrochalcone, saccharin and sucrose are summarised in Table 4.

Table 4. Cost comparisons between neohesperidin dihydrochalcone, saccharin and sucrose.

Compound	Sweetness	Cost per kg. (1972)	Weight sweetener*	Equivalent costs†
Neohesperidin dihydrochal-cone.	1000x sucrose	£70.00	1g	7p
Saccharin	500x sucrose	£1.358	2g	0.27p
Sucrose	÷	9.4p.	1kg	9.4p

* Weight sweetener providing a sweetness equivalent to 1kg sucrose

† Cost of sweetener equivalent in sweetness to 1kg sucrose.

If a sufficient quantity of grapefruit were available for naringin extraction then neohesperidin dihydrochalcone could be manufactured in a quantity sufficient to replace half the saccharin which is manufactured at present. The major obstacle is the currently high cost of naringin, although the cost of manufacturing neohesperidin dihydrochalcone would decrease if the amount of peel processedvariedgreatly and if the process could be improved (eg. if the isovanillin condensation were to be carried out directly on the alkaline peel extract followed by a direct hydrogenation.)

It is suggested that the use of neohesperidin dihydrochalcone could be attractive in those situations where sugar cannot be used for technical reasons. It is possible to achieve sweetness at approximately 75% of the cost of sucrose.

2. Occurrence

Dihydrochalcones

Eleven compounds with the dihydrochalcone structure have been found to occur in nature. Phloretin (30) is the most important and it occurs as the 2'-β- D - glucoside, phloridzin (31) in most of the twenty-five species of Malus (Rosaceae) [9,18] listed by Rehder [11] such as Pyrus and Prunus. Phloridzin was first isolated by de Koninck[12] in 1835 from the root bark of the apple tree. This author also reported that phloridzin was present in the root bark of pear, cherry and plum trees but it is now recognised[13] that phloridzin is not present in any significant amounts in the latter species although it is certainly present in apple trees. In fact, the root bark of the apple tree still provides the richest source of this compound.

Although phloridzin is characteristic of the genus Malus there are exceptions. Thus, phloridzin is replaced either wholly or in part in certain species by phloretin - 4' -β - D - glucoside (32). For example, phloridzin is entirely absent from the plant Malus trilobata but phloretin - 4' -β - D - glucoside is present[14] in the case of four species of Series 3, Sieboldianae[13,14] (see Rehder[11]). Phloridzin is replaced by 3 - hydroxyphloretin - 4' -β - D - glucoside (34) Phloretin, accompanied by its 4' - methyl ether (asebogenin (35)) has also been found in Ericaceae (Kalmia and Pieris)[15] and an ecological form of Andromeda japonica has also been shown to contain phloridzin[16]. Phloretin has also been isolated from another unrelated source - Smilax glycyphylla in the Liliaceae[15].

Asebotin is a toxic compound which was first recovered from Pieris japonica by Eykman[17] and from Kalmia latifolia by Bourquelot[18]. Its structure is known to be 2; 4, 6'- trihydroxy - 4' - methoxy-dihydrochalcone - 2' -β- D - glucoside.[19,20,21,22]. Williams has reported[9] that whilst Kalmia latifolia contains phloridzin Kalmia angustifolia contains asebotin but no phloridzin. Kalmia polifolia contains neither of these compounds. In the case of the Pieris genus; Pieris japonica contains phloridzin but no asebotin, Pieris taiwanensis contains asebotin but no phloridzin, Pieris floribunda contains neither compound but the garden varieties of Pieris contain both.

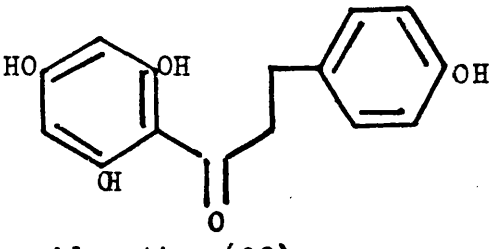
Glycyphyllin (32) the sweet principle of the Australian medicinal plant Smilax glycyphylla is a closely allied substance to phloridzin and according to Rennie[23] is a phloretin rhamnoside. Its structure has been shown to be phloretin - 2' -α- L - rhamnoside[22]. This substance was found to be present in a sample of leaves from a Smilax glycyphylla plant obtained from New South Wales, Australia but samples of the same species obtained from Queensland and Kew Gardens, London contained no glycyphyllin[9]. This substance is possibly the only sweet dihydrochalcone known to be naturally occurring. A sample was tasted by Horowitz[24] and found to have a bitter sweet taste of moderate intensity but the bitterness was predominant.

A phloretin arabinosylglucoside and a phloretin xylosylglucoside are also present in Malus but they were detected chromatographically and insufficient material was recovered for a detailed structural determination[25].

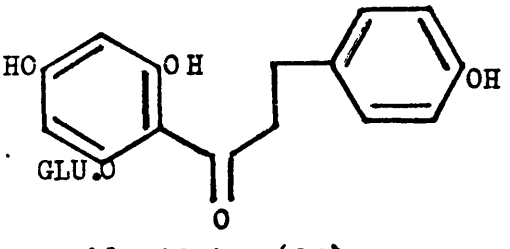
There are two naturally occurring dihydrochalcone aglycones. Firstly, the buds of the balsam poplar (Populus balsamifora) contain a methylated deoxy-phloretin[26] and secondly the fronds of a silver fern which contain both the compound obtained from the poplar and another methylated phloretin[27].

It is evident, therefore that dihydrochalcones are not widely distributed throughout the plant kingdom and that if sweet dihydrochalcones are ever to be marketed as sweetening agents their source will lie either in naturally occurring chalcones and flavanones or they will be made synthetically.

The naturally occurring dihyrochalcones and their sources are summarised in Table 5. Their structures are shown below:



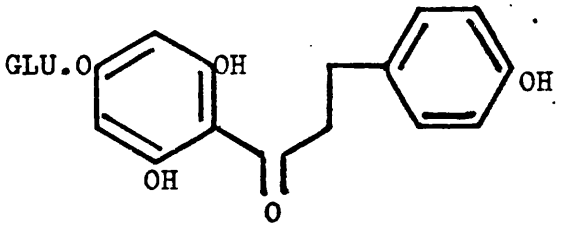
phloretin (30)



phloridzin (31)

TABLE 5. Naturally occurring dihydrochalcones

Compound No.	Compound	Substituents	Sources	Ref
(30)	Phloretin	2',4,4',6'-tetrahydroxy	Smilax glycyphylla, Kalmia & Pieris (Ericaceae)	15
(31)	Phloridzin	phloretin-2'-β-D-glucoside	Andromeda japonica Malus (Rosaceae)	9,10,12, 13,14
(32)	Glycyphyllin	phloretin-2'-β-D-rhamnoside	Smilax glycyphylla	22,23,24
(33)	Phloretin-4'-β-D-glucoside		Malus trilobata	14
(34)	3-hydroxy phloretin-4'-β-D-glucoside		Sieboldianae	13,14
	Phloretin arabinosyl glucoside		Malus	25
	Phloretin xylosyl-glucoside		Malus	25
(35)	Asebogenin	2',4,6' trihydroxy-4'methoxy	Kalmia & Pieris (Ericaceae)	15
(36)	Asebotin	asebogenin-2'-β-D-glucoside	Andromeda japonica (Pieris japonica), Kalmia latifolia,	9,17,18, 19,20,21, 22
(37)	2'6'-dihydroxy-4'-methoxydihydrochalcone?		Populus balsamifera	26
	a methylated phloretin			27



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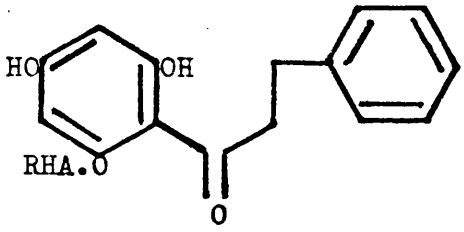
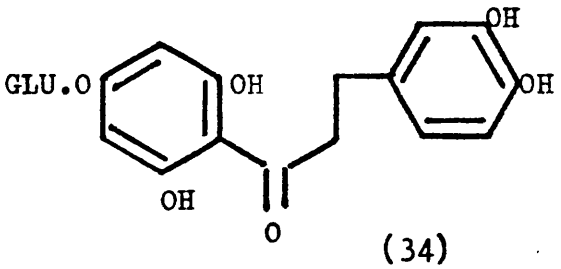


glycyphyllin (32)



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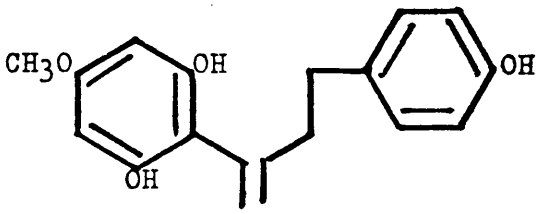
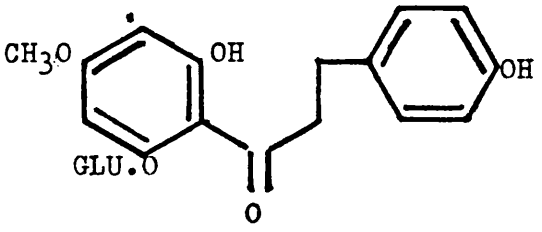
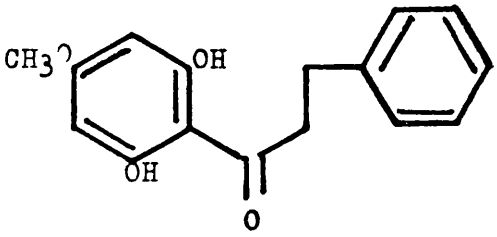


asebogenin (35)



asebotin (36)



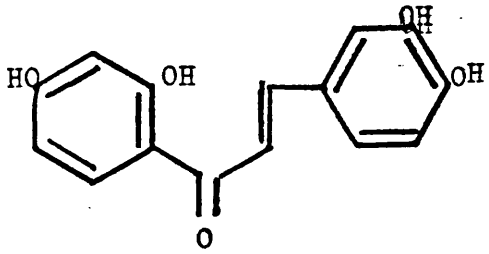
(37)

Chalcones

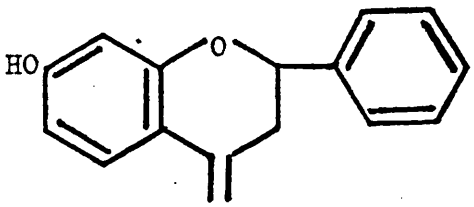
Only a few naturally occurring chalcones are known but they occur with some frequency in the Compositaes being present in twelve genera, notably in Coreopsis and Dahlia. Chalcones are also present in several Leguminosae (Butea, Cylicodiseus, Glycyrrhiza, Plathymenia, Ulex), and in Didymocarpus (Gesneriaceae). A more complete list may be found in "The Chemistry of Flavonoid Compounds" edited by T.A. Geissman[28].

It was Gertz[29] who showed that a large number of species of Compositae contained "anthochlor" pigments. (Anthochlor being the collective name for two groups of pigments, chalcones and aurones. Both these classes of compound give red or orange colourations with alkali). The first example of a typical anthochlor pigment was that of the chalcone butein(38) considered by Price[30] to be present as an unknown glucoside in a yellow variety of Dahlia.

Butein was first obtained by Perkin and Hummel[33] in the course of isolating butin(39) (the isomeric flavanone) from the flowers of Butea frondosa. Until quite recently it was believed that the butein which was isolated from Butea flowers might have arisen from the isomerisation of butin in the isolation process.

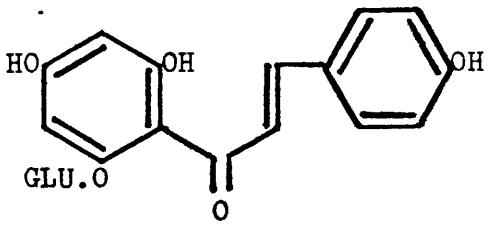


butein (38)

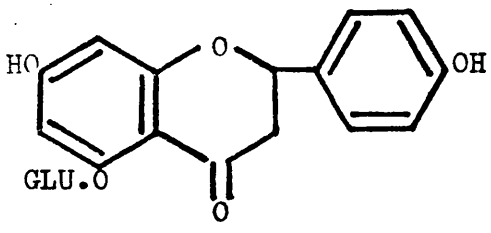


butin (39)

Geissman has proved[32], however, that butein is actually present in Coreopsis douglasii flower and that the flavanone butin is absent. This finding supports Perkin's claim to have isolated both the chalcone and flavanone as does the existence of several other chalcone/flavanone pairs co-existing in a number of plants. These pairs are resorcinol-derived flavonoids or compounds which have no free hydroxyl groups in the 2' and 6' positions of the chalcones. Consequently, in the absence of a hydroxyl group in the 5 - position of a flavanone the co-occurrence of chalcone and flavanone is possible. The phenomenon of an hydroxyl group in the 5 - position of a flavanone conferring stability by hydrogen bonding on flavanones is discussed below. The co-existence of salipurposide(41) (a flavanone) and isosalipurposide(40) (a chalcone) in the bark of Salix purpurea has long been known[33,34] and the yellow chalcone isoliquiritin(42)

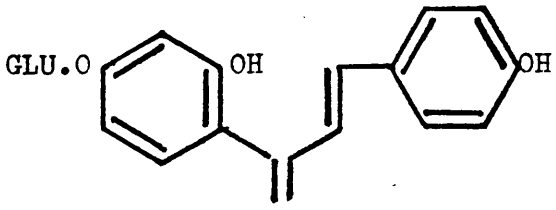


isosalipurposide (40)

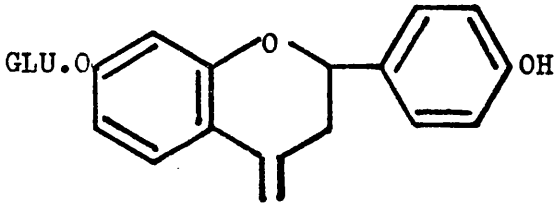


salipurposide (41)

has been isolated along with liquiritin(43) from dried liquorice roots[35,36]. It was also found that fresh liquorice roots contain only the chalcone dervative.



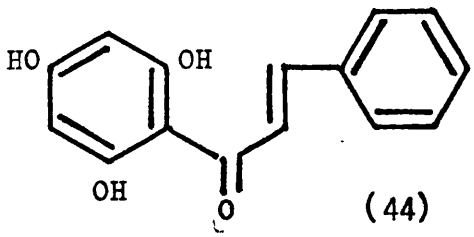
isoliquiritin (42)



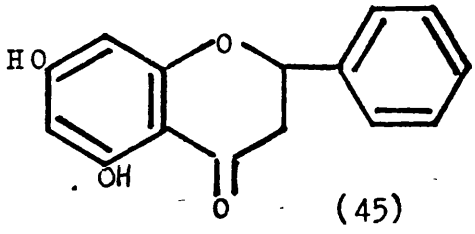
liquiritin (43)

The chalcone glycosides are usually substituted at the 4'- position (corresponding to the 7- position of flavanones) or less frequently at the 2'- position (corresponding to the 5- position of flavanones).

Whereas the majority of naturally occurring flavanones have a phloroglucinol derived structure in ring A no naturally occurring chalcones are known to possess this structure or that of a 2', 6' - dihydroxy structure. Thus, a 2', 4', 6' - trihydroxy chalcone compound(44), for example, would easily isomerise to a 5,7 - dihydroxy- flavanone, compound(45), for example.

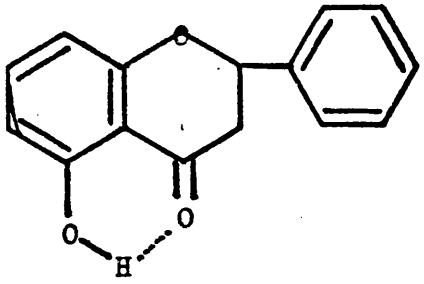


(44)

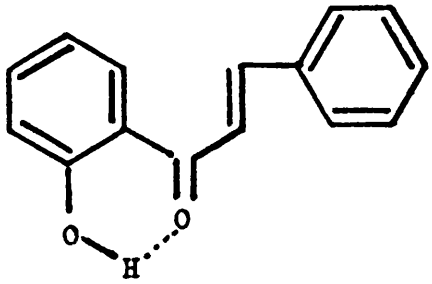


(45)

Seshadri and Narasimhachari[35,37] found that naringenin, isosakuranetin, naringenin 4',7 - dimethyl ether etc. dissolve readily in cold 10% sodium hydroxide and are precipitated unchanged by acidification whereas the 5 - methoxyl and 5, 7 - dimethoxyl flavanones dissolve in this reagent only on warming and give only the corresponding 2' - hydroxychalcones on acidification. From these experiments they pointed out that when the 5 - hydroxyl group is present in the flavanone the chalcone-flavanone isomerisation is strongly on the side of the flavanone because the ring is stabilised by hydrogen-bonding:



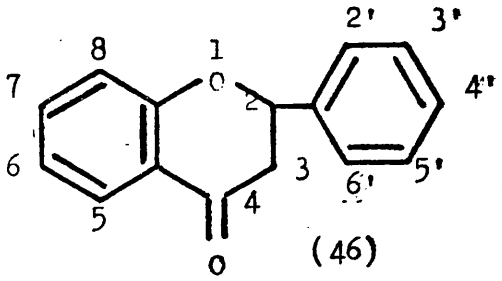
In a 2' - hydroxy chalcone such bonding favours a configuration in which the olefin bond is thrust away from the hydroxy group so that cyclisation is opposed:



There is no justification for the generalisation sometimes made that 5 - hydroxyflavanones do not suffer ring-scission- they do, but it is true that the chalcones are not easily isolated. Conversely, syntheses aimed at the preparation of a 2'6' - dihydroxy-chalcone give flavanones instead because the hydrogen-bonding of one hydroxyl group merely favours cyclisation at the other. Etherification of either hydroxyl group nullifies the effect and chalcones can then be obtained easily.

Flavanones.

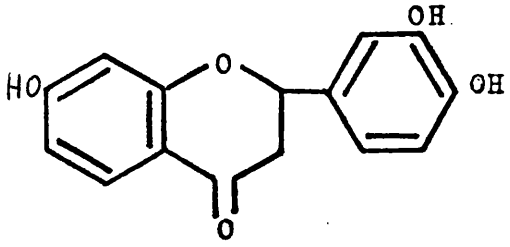
Flavanone (46) is a colourless substance which has not as yet, been found to exist in nature in the free state.



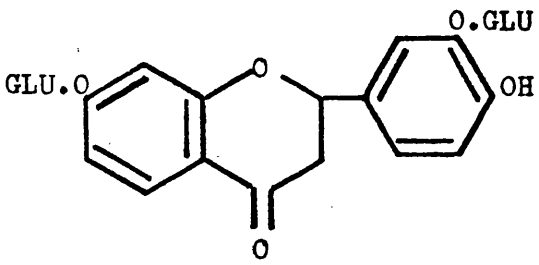
(46)

About fifty hydroxylated flavanones, however, occur either in the free form or in combination as glycosides in flowers, fruits, bark and roots. They appear to be of fairly general distribution especially in higher plants such as Rosaceae, Rutuceae, Gluminosae, Compositae, Hydrophyllaceae, Pinaceae and in the fern family - Polypodiaceae.

Butin (47) was the first compound to which was ascribed the correct flavanone structure. It was isolated as butrin(48), the 3', 7-diglucoside of butin from the flowers of Butea frondosa[38].

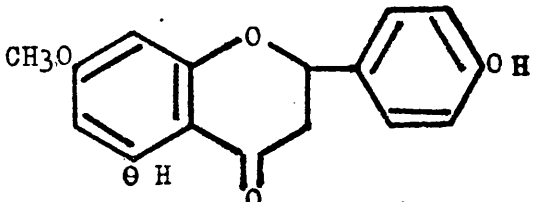


butin (47)

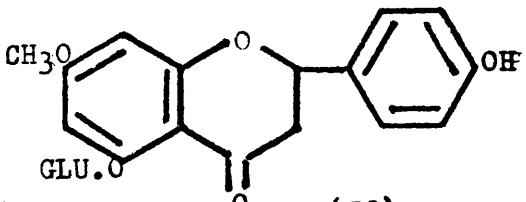


butrin (48)

After Asahina and co-workers had determined the structure of sakuranetin[39] (49) which was present in the bark of Prunus yedoensis and Prunus speciosa as the glucoside sakuranin (50) such compounds as hesperidin and naringenin were found to be flavanones.

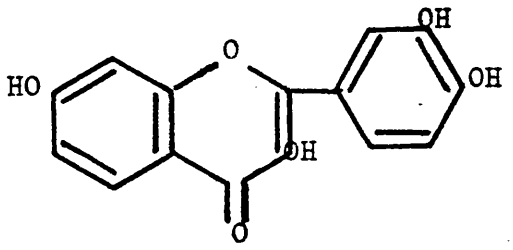


sakuranetin (49)

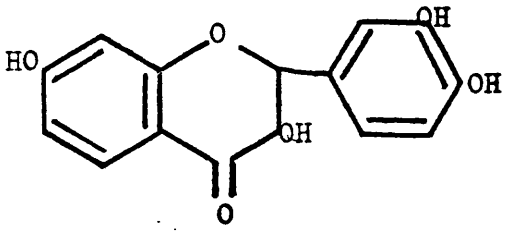


sakuranin (50)

Flavanones can be regarded as 2,3-dihydro derivatives of the flavones. In fact, it is not uncommon to find a flavone and a flavanone co-occurring side by side in the same plant. Thus, the flavone fisetin(51) is found[40] with the flavanone fustin(52).



fisetin (5I)



fustin (52)

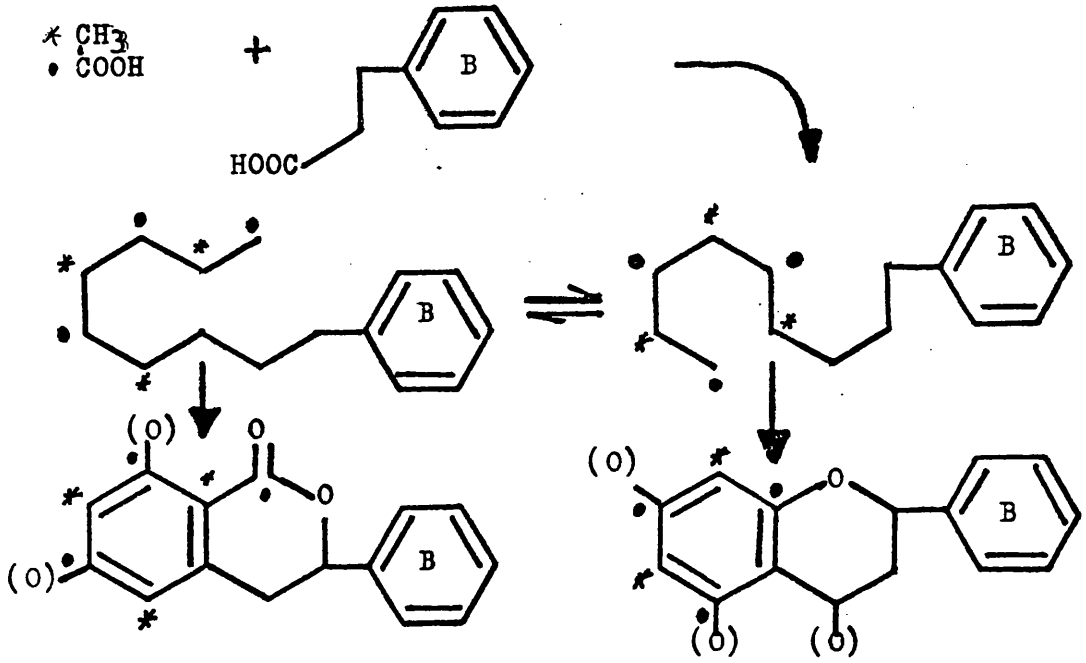
Flavanones are associated more closely than the flavones with heart woods, barks and roots and less so with the leaves and petals. That is, the state of oxidation of flavonoids increases as the upper extremities of the plant are reached.

Lists of naturally occurring flavanones and their sources may be consulted[28,41].

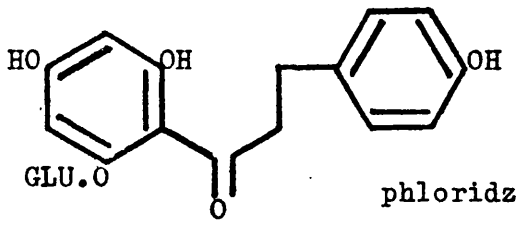
3) Biochemistry

It has been shown by feeding ^{14}C-labelled precursors to plants that a number of compounds having two aromatic rings in their structures form one of these rings from activated acetate units and the other via the shikimic acid pathway. Flavanoids come into this category. This result was predicted by Robinson[42] and by Birch and Donovan[43] from a consideration of the positions of the oxygen atoms in most flavonoids. One ring commonly has the phloroglucinol or resorcinol pattern and the other a vicinal di-or-tri-oxygen substitution. These compounds are formed by addition of three activated acetate units to a phenyl propanoic acid. Ring closure may occur in at least two different ways, as shown in Fig I, to give a flavonoid or, less frequently, an isocoumarin. The activated acetate units are probably in the form of malonyl-CoA and the phenyl-propanoic acid is probably a cinnamic acid derivative, possibly a CoA ester.

Fig. I. General scheme for biosynthesis of aromatic compounds of mixed origin.



Hutchinson et al[44] have studied the bisynthesis of phloridzin(31) in leaf discs of Malus, by feeding ^{14}C-labelled precursors by vacuum infiltration. Both acetate and phenylalanine were precursors of phloretin, the phenylalanine being incorporated into ring "B" and the acetate into ring "A". Avadhani and Towers[45] showed that [α-^{14}C] cinnamic acid was also readily incorporated into phloridzin.



phloridzin (3I)

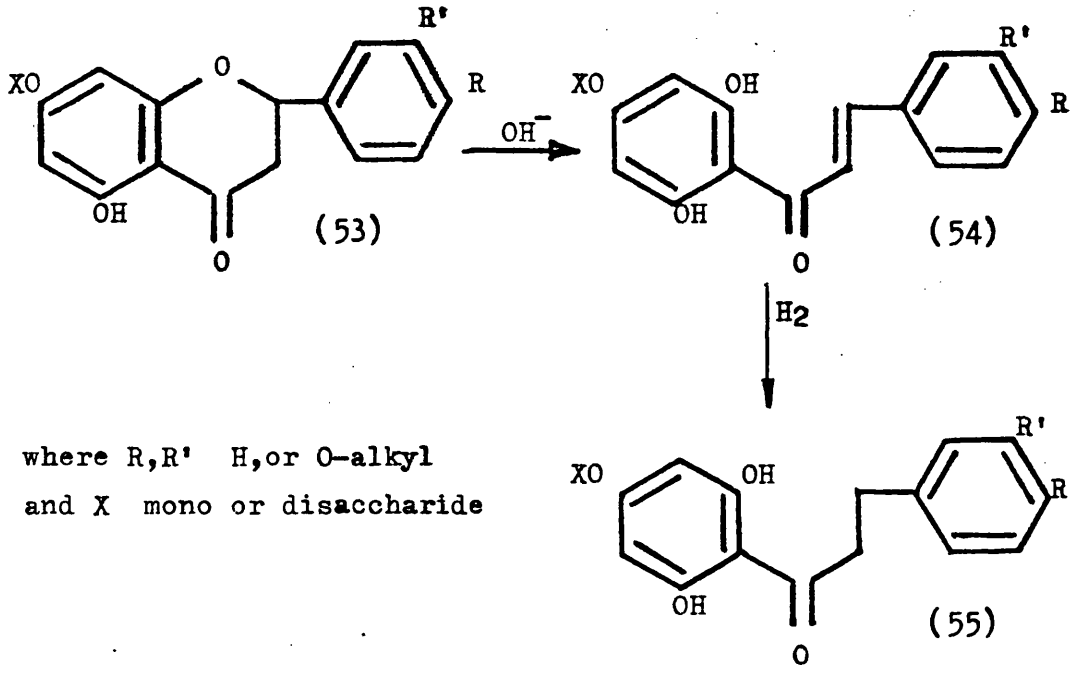
The mechanism for the synthesis of aromatic compounds via the shikimic acid pathway has been established mainly as a result of the work of Davis who has reviewed this work[46]. Sprinson has also produced a review.[47]

4) Synthesis

The synthesis of dihydrochalcone glycosides can be attempted by several different approaches:

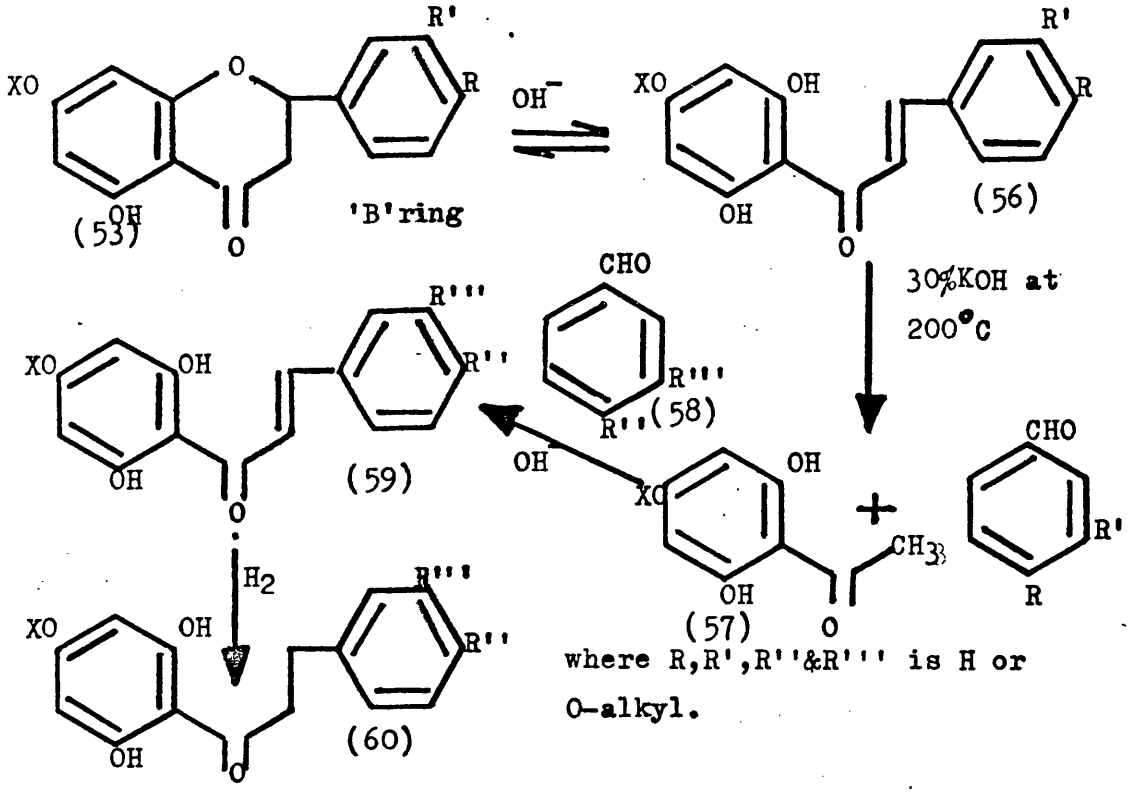
Route A. From naturally occurring flavanones or chalcones.

A chalcone(54), which can be either naturally occurring or, alternatively, which can be obtained from a naturally occurring flavanone(53) by treatment with alkali, is hydrogenated to yield a dihydrochalcone(55).



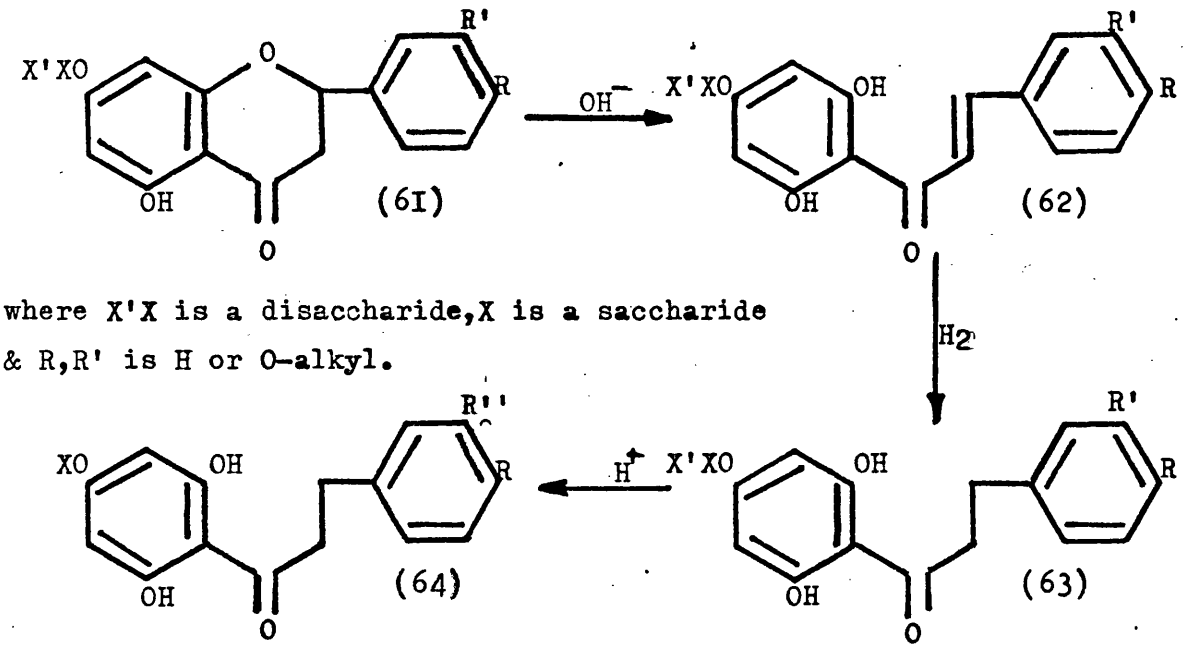
Route B. By modification of naturally occurring flavanones or chalcones.

a) 'B' ring modification If a flavanone(53) (naturally occurring chalcone can also be used) is treated with alkali a chalcone is formed(56) which on heating to an elevated temperature may undergo alkaline hydrolysis to the ketone(57). This ketone may undergo a Claisen-Schmidt condensation with variously substituted aldehydes(56) to form a new chalcone(59) and on hydrogenation, a new dihydrochalcone(60).



b) Disaccharide Hydrolysis to a Monosaccharide.

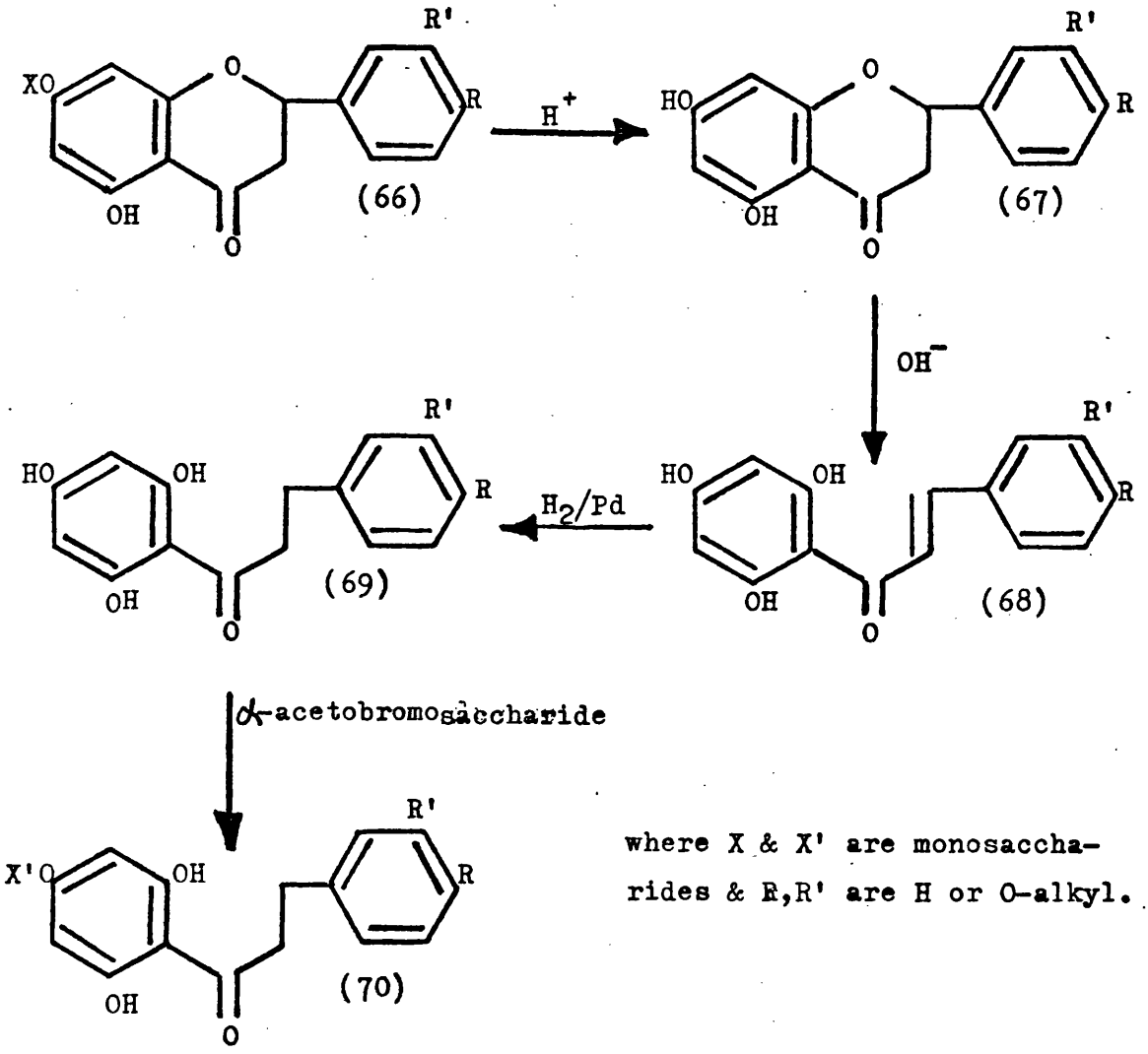
A flavanone disaccharide(61) is converted to its chalcone(62) or a naturally occurring chalcone disaccharide is used and hydrogenated to its dihydrochalcone(63). The dihydrochalcone disaccharide is then hydrolysed to its dihydrochalcone monosaccharide(64) by boiling in a dilute acid.



Some dihydrochalcone aglycone(65) will also be formed.

c) Glycosylation of a dihydrochalcone aglycone obtained from a naturally occurring flavanone or chalcone glycoside.

A flavanone glycoside(66) is hydrolysed to its aglycone(67), converted via its chalcone(68) to a dihydrochalcone(69) and glycosylated to a dihydrochalcone glycoside(70) using an α-acetobromosaccharide.

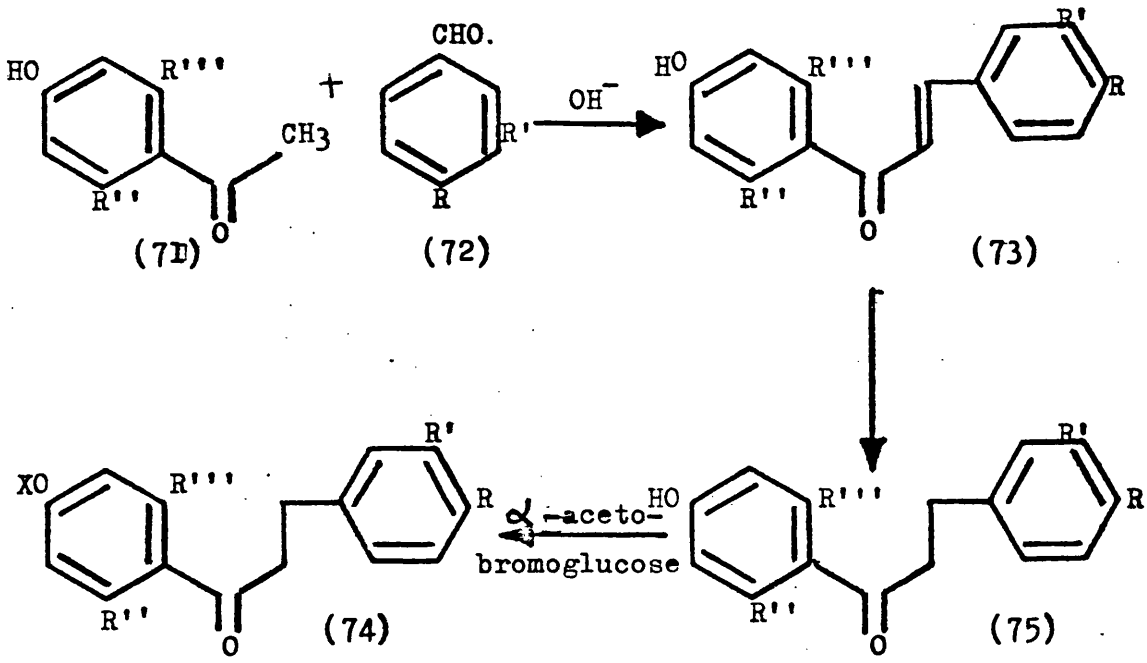


where X & X' are monosaccharides & R,R' are H or O-alkyl.

Route C. From simple starting materials.

a) Glycosylation of an aglycone.

A dihydrochalcone may be prepared by reaction of an aromatic ketone (71) with an aromatic aldehyde(72) by a Claisen-Schmidt type condensation in the presence of strong alkali to form a chalcone(73) which can be hydrogenated to a dihydrochalcone aglycone(75). This aglycone may be glycosylated using an α- acetobromosaccharide to a dihydrochalcone glycoside(74).

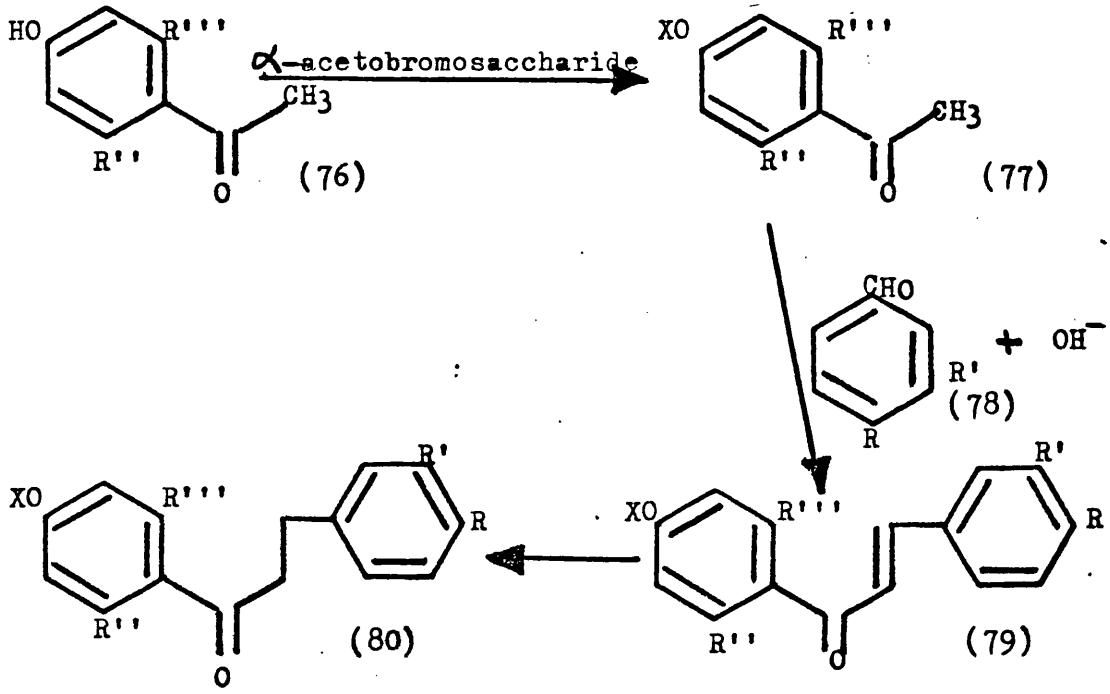


where R,R',R'',R''' is H or alkoxy.

X is a saccharide.

b) Glycosylation of a ketone followed by dihydrochalcone formation.

A ketone glycoside(77) is prepared by reaction of an α- acetobromosaccharide with a substituted ketone(76) which is then reacted with a substituted aromatic aldehyde(78) to form the chalcone glycoside(79). The chalcone can be hydrogenated to the dihydrochalcone glycoside(80).

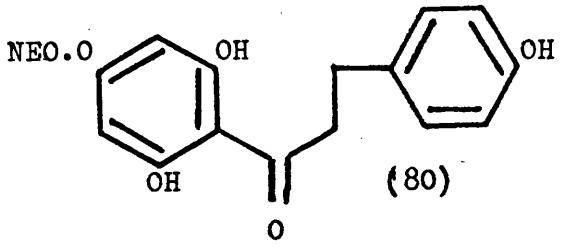


where R,R',R'',R''' is H or alkoxy.

X is a saccharide.

Some examples of the synthesis of dihyrochalcone glycosides can be found in the literature and are outlined below.

Horowitz and Gentili prepared three sweet dihydrochalcones by converting naturally occurring flavanones into dihydrochalcones via their chalcones[48]. i.e. by Route A. By this means, they prepared naringin dihydrochalcone(80) neohesperidin dihydrochalcone(81) and prunin dihydrochalcone(82)



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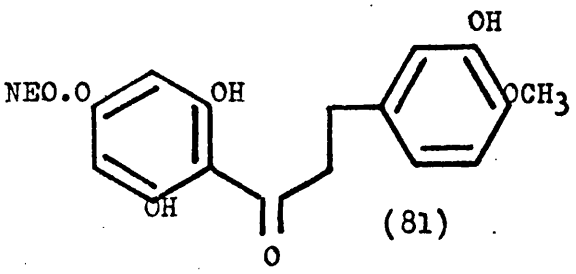

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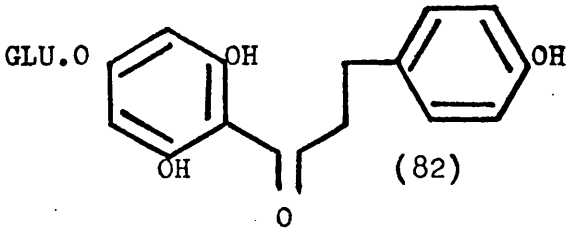
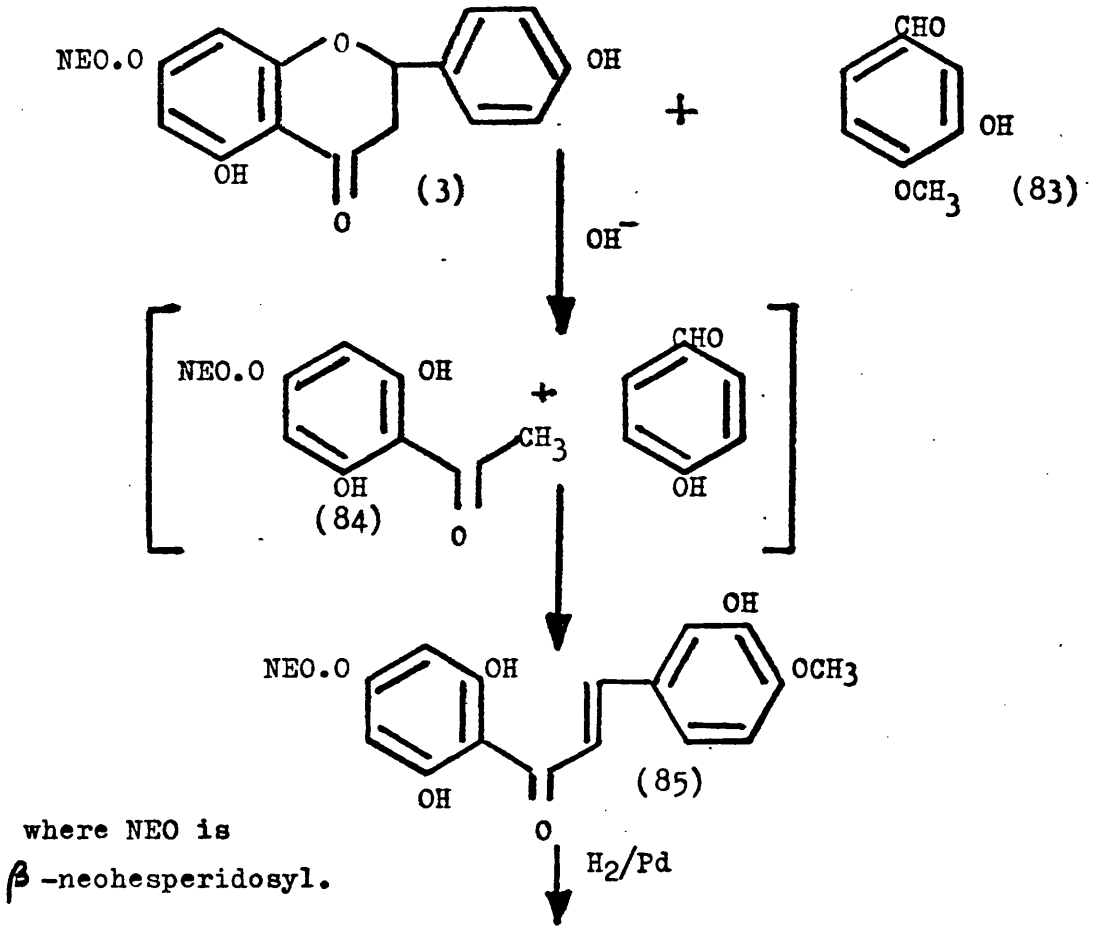


(82)

where NEO is β-D-neohesperidosyl & GLU is β-D-glucopyranosyl.

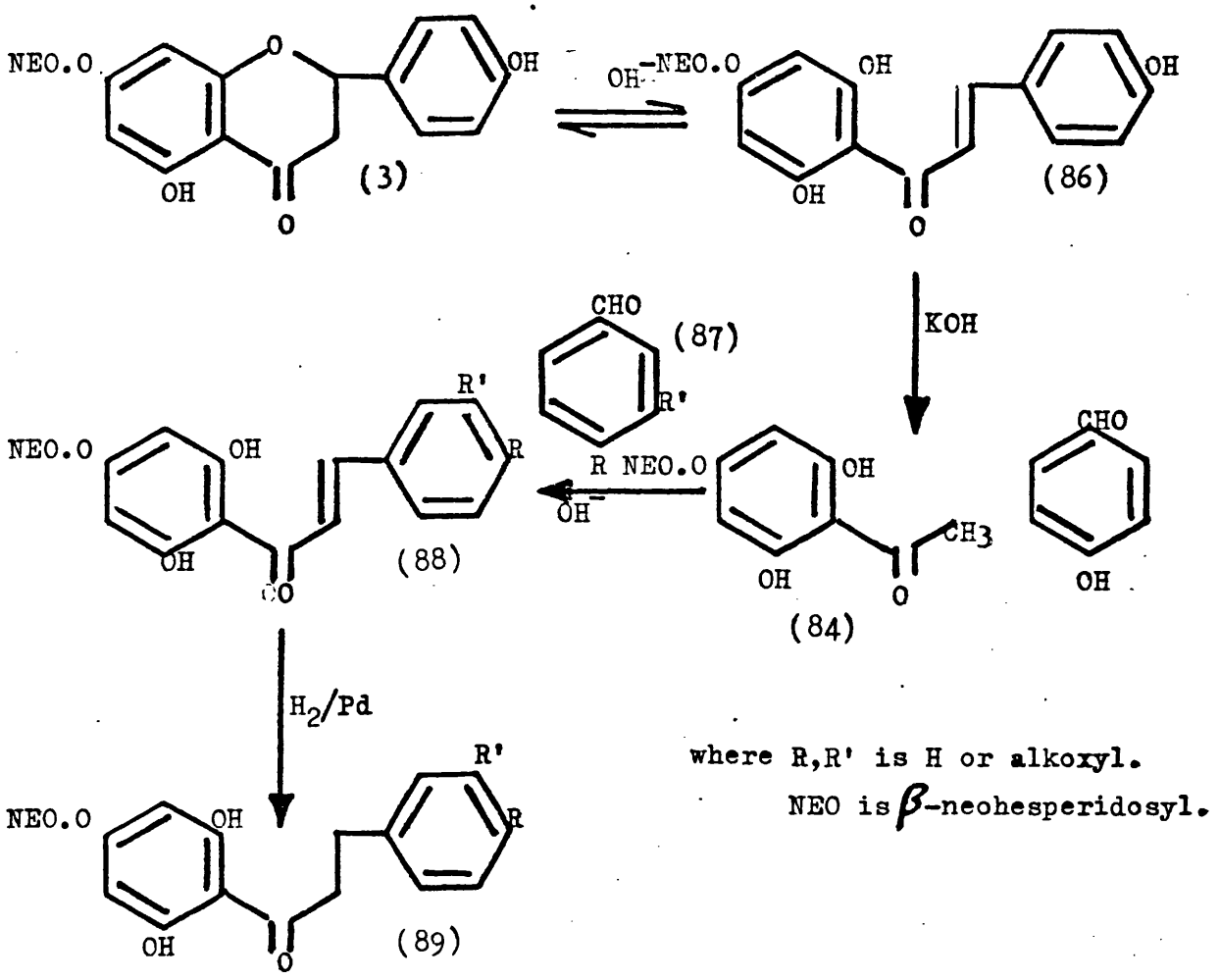
In 1968, Horowitz and Gentili published a second patent[49] in which they described their preparation of neohesperidin by reacting together naringin(3) and a twelve - molar excess of isovanillin(83) in 10 to 25% potassium or sodium hydroxide at 50 to 100°c for four and a half hours. The resulting neohesperidin chalcone(85) was purified and then hydrogenated to neohesperidin dihydrochalcone(81). In this method, the intermediate hydrolysis product, phloro-acetophenone- 4' -β- neohesperidoside(84) is not isolated but is reacted in situ with excess isovanillin, tending to drive the reaction to completion. The authors found that the IR and NMR spectra of the product were indistinguishable from those of natural neohesperidin and free of any naringin dihydrochalcone contaminant.



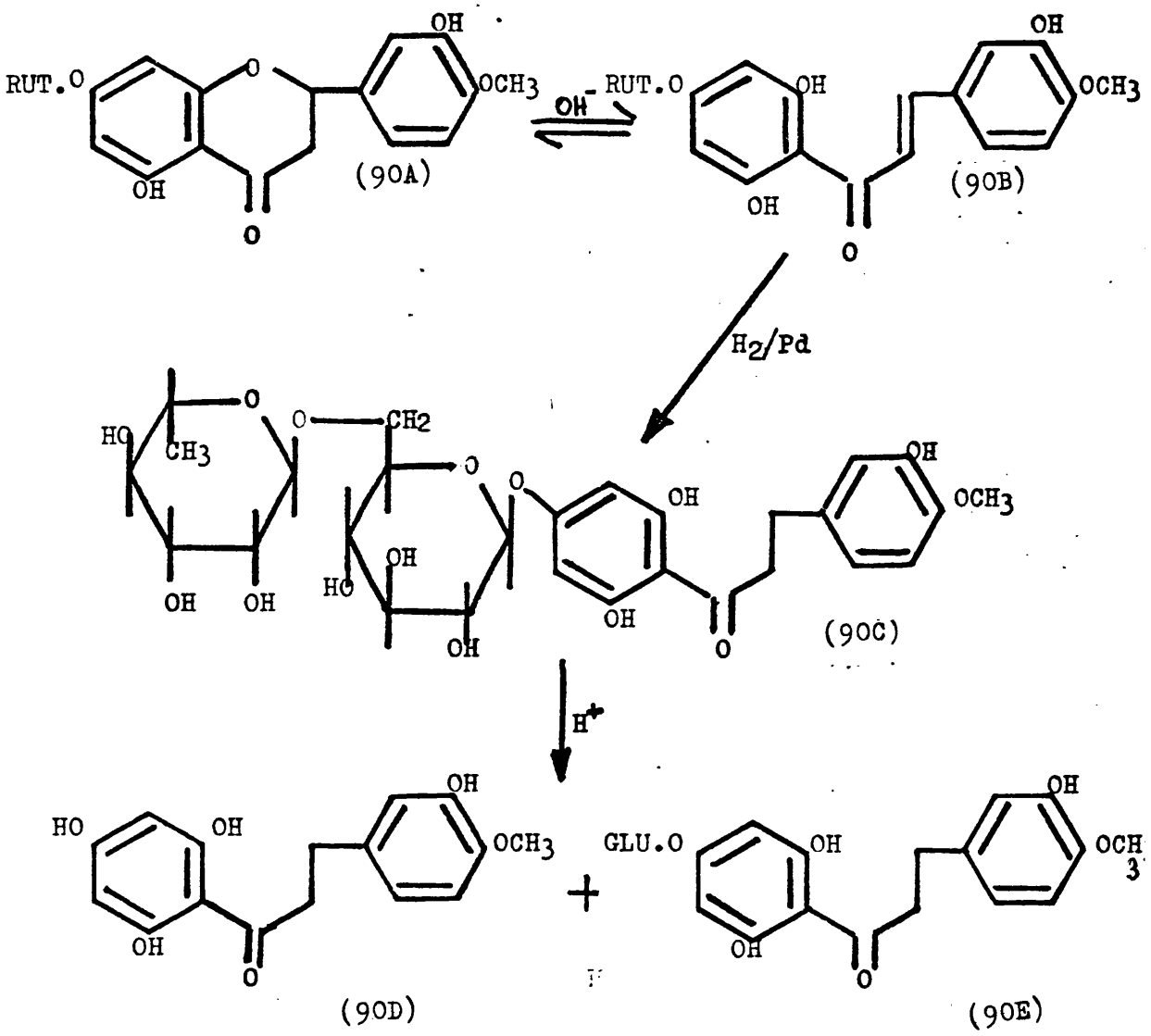
where NEO is β-neohesperidosyl.

Neohesperidin dihydrochalcone (81)

Krbechek, Inglett et al. described[4] their preparation of several sweet dihydrochalcones in 1968. These authors subjected naringin(3) to alkaline hydrolysis in order to prepare phloroacetophenone - 4' - β - neohesperidoside(84). They carried out Claisen-Schmidt condensations by reacting this compound with various substituted benzaldehydes(87) to yield, firstly the chalcones(88) and secondly, following hydrogenation of the chalcones, various substituted dihydrochalcones(89).

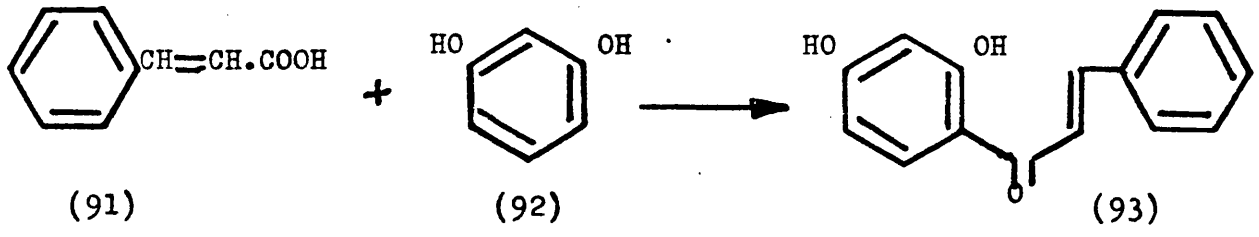


Horowitz and Gentili published(50) a third patent in 1969 in which they describe the preparation of hesperetin dihydrochalcone glucoside(90E)from hesperidin(90A)a flavanone glycoside which occurs naturally in oranges and lemons and is a by-product of the commercial processing of such fruit. This preparation was achieved by reacting the tasteless starting material, hesperidin(90A)with a 10 to 25% aqueous solution of sodium or potassium hydroxide at room temperature in order to form hesperidin chalcone(90B). The chalcone was converted into the virtually tasteless hesperidin dihydrochalcone(90C) by hydrogenation and this compound was converted into hesperetin dihydrochalcone glucoside by a hydrolytic technique using an acid solution of between 0.01 M and 1 M concentration and boiling the solution under reflux for 2.50 to 2-75 hours. The reaction mixture was shown to contain a 50:50 mixture of hesperetin dihydrochalcone(90D) and hesperetin dihydrochalcone glucoside(90E)with virtual absence of hesperidin dihydrochalcone starting material.

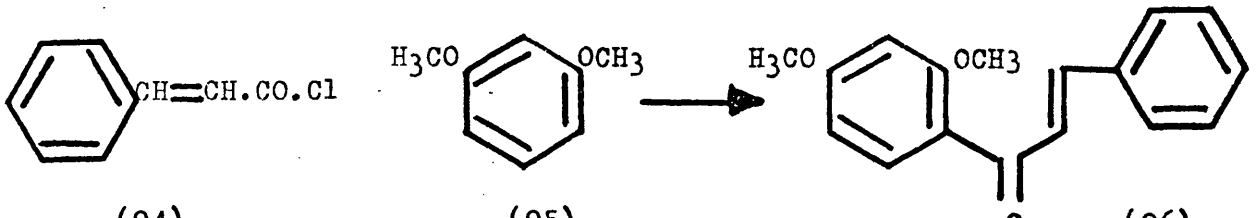


Dihydrochalcone Glucoside Synthesis.

The simplest method used for the preparation of dihydrochalcones is that of preliminary chalcone formation by a Claisen-Schmidt condensation followed by hydrogenation (see page22). Two other methods have been described in the literature thus, an $\alpha\beta$- unsaturated acid (91) can be reacted with a phenol[51](92) to yield a chalcone(93) which could be hydrogenated to the dihydrochalcone.

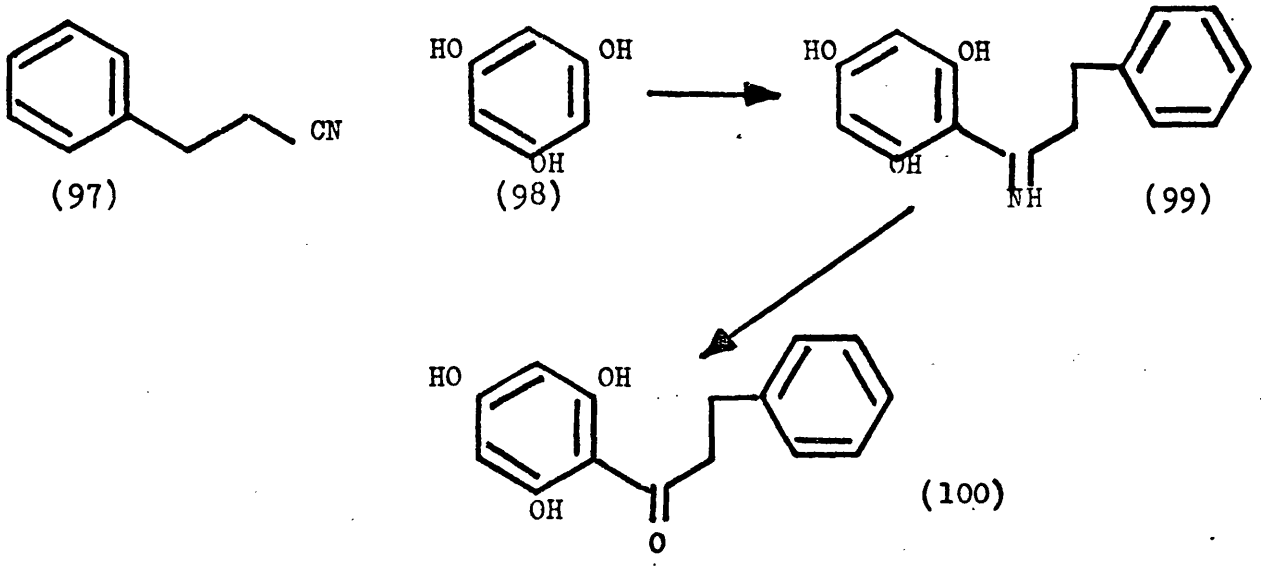


Also, an $\alpha\beta$- unsaturated acid chloride(94) can be reacted with an appropriate aromatic molecule[52](95) to yield a chalcone(96) which could be hydrogenated to the dihydrochalcone.



(94) (95) (96)

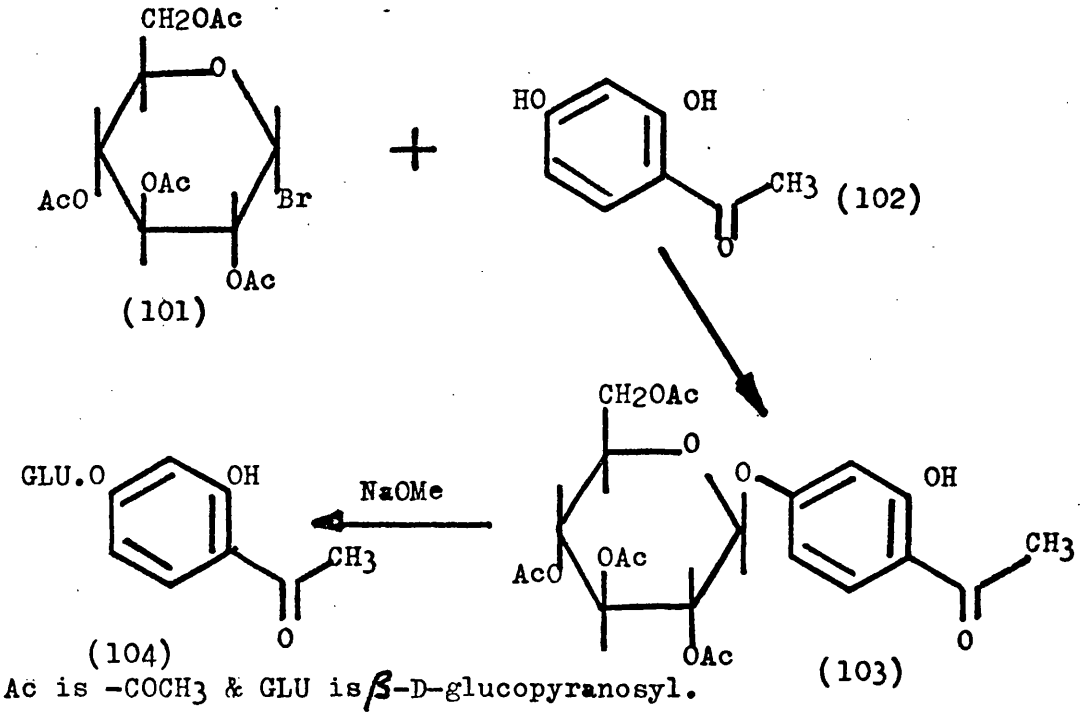
A direct route to the dihydrochalcones would make use of the Hoesch reaction[53]. No reports of this application of the Hoesch have been seen in the literature. A cyanide(97) would be reacted with a phenol(98) in the presence of anhydrous zinc chloride and with a stream of dry hydrogen chloride gas being passed through the reaction mixture. An imide is first formed(99) which could be hydrolysed to the dihydrochalcone(100) by boiling in aqueous solution.



(97) (98) (99) (100)

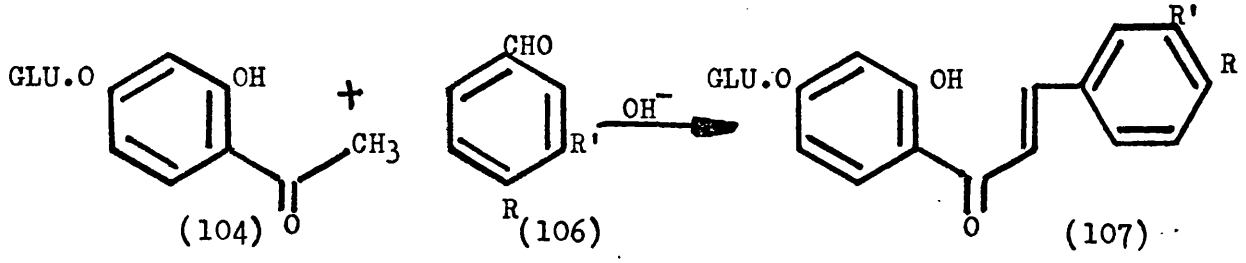
The dihydrochalcone aglycones(93,96,100) prepared by these three methods would be glycosylated by reaction with α- acetobromoglucose. (See page 22).

Several authors have prepared chalcones starting with resacetophenone - 4' - (1-O-β - D - gluco-pyranoside) (104) a compound which is prepared by reaction of resacetophenone(102) with α - acetobromo-glucose(101). Reichel prepared resacetophenone - 4' - (1 - O -β- D - tetraacetylglucopyranoside) (103) and deacetylated it to the glucoside (104) by adding sodium methoxide[54].

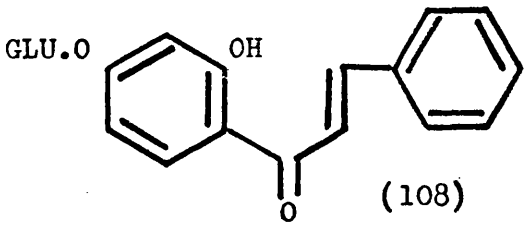


where Ac is $-COCH_3$ & GLU is β-D-glucopyranosyl.

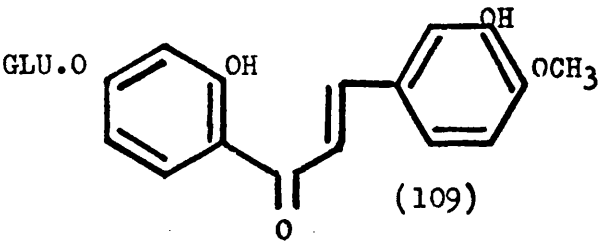
Reichel then prepared chalcones by reaction of the resacetophenone - 4' - (1- O -β - D - glucopyranoside) (104) with either a substituted benzaldehyde(106) (benzaldehyde or isovanillin) to form a chalcone(107).



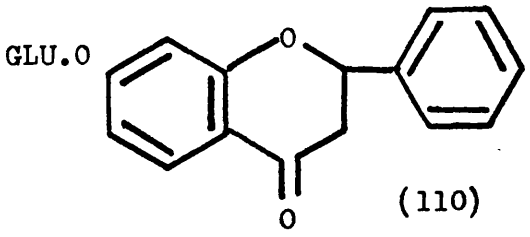
By this means he prepared 2',4' - dihydroxychalcone - 4' (1-O-β- D - glucopyranoside) (108) and 2',3,4' - trihydroxy - 4 - methoxychalcone - 4'- (1-O-β - D - glucopyranoside)(109) and the flavanones corresponding to these chalcones, that is, 7-hydroxy flavanone - 7 - (1-O-β - D - glucopyranose(110) and 3', 7- dihydroxyflavanone - 7 - (1-O-β- D-glucopyranose(111).



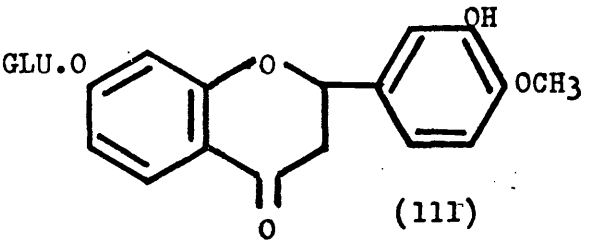
(108)



(109)

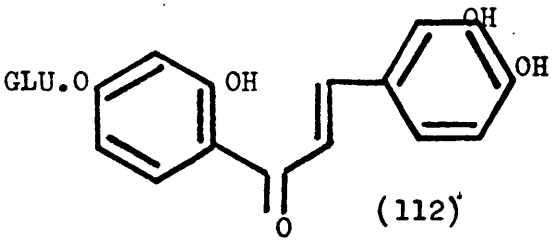


(110)



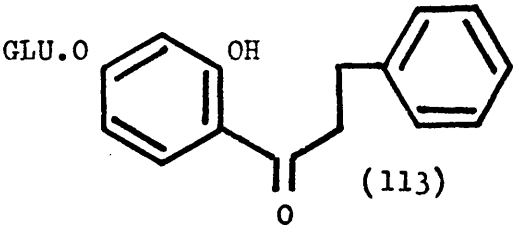
(111)

Coreopsin (112), a glucoside of butein, has been prepared by Geissman[55], Shimokoriyama[56], Nordstrom[57], Puri[58], Farkas and Pallos[59] and Mauthner[60].

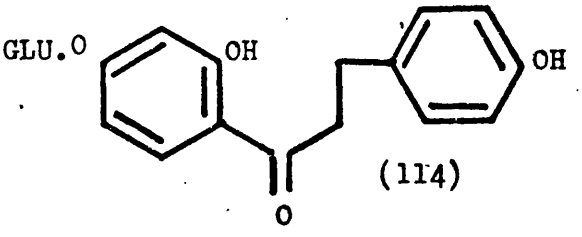


(112)

Bognar and Farkas[61,62] reacted resacetophenone tetraacetylglucoside with benzaldehyde in 60% potassium hydroxide for a day at room temperature to form 2',4' - dihydroxychalcone - 4' - (1-O-β- D- glucopyranoside)(108) which they hydrogenated to the dihydrochalcone(113).



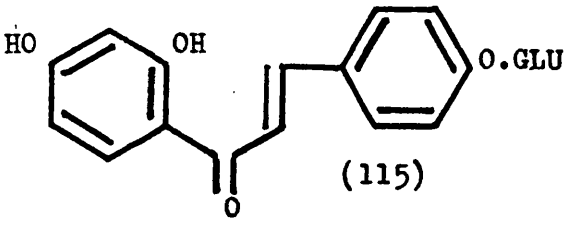
(113)



(114)

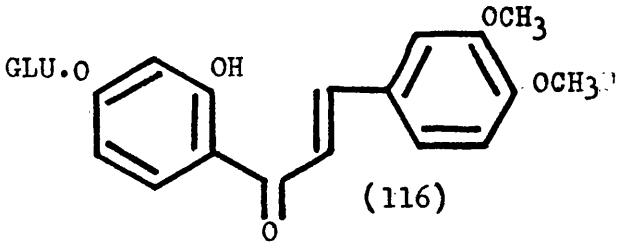
Jorio[63] also reacted resacetophenone - 4' - (1-O-β-D- glucopyranoside with 4 - hydroxybenzaldehyde in alkali and obtained the chalcone i.e. 2',4, 4' - trihydroxychalcone - 4' - (1-O-β-D- glucopyranoside) after three days standing at room temperature followed by acidification. The chalcone was hydrogenated to the dihydrochalcone (114).

Litvinenko[64] has also prepared 2',4,4'- trihydroxychalcone - 4' - (1-O-β-D- glucopyranoside) but in addition has also prepared 2',4,4' - trihydroxy-chalcone - 4 - (1-O-β - D- glucopyranoside)(115)

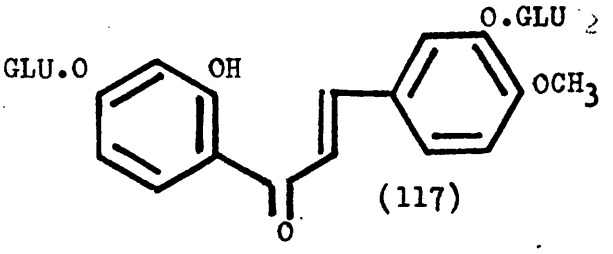


(115)

The synthesis of glucochalcones by glucosidation of a chalcone aglycone was accomplished by Mauthner[65]. The reaction of resacetophenone and veraltraldehyde resulted in the formation of 2',4' - dihydroxy - 3,4 - dimethoxy - chalcone which was in turn reacted with α - acetobromoglucose in quinoline with silver oxide catalyst to yield 2',4' - dihydroxy - 3,4 - dimethoxychalcone - 4' - (1-O-β- D - tetraacetylglucopyranoside). This compound after deacetylation by treatment with barium hydroxide gave 2', 4' - dihydroxy - 3,4 - dimethoxychalcone - 4' - (1-O-β-D - glucopyranoside).

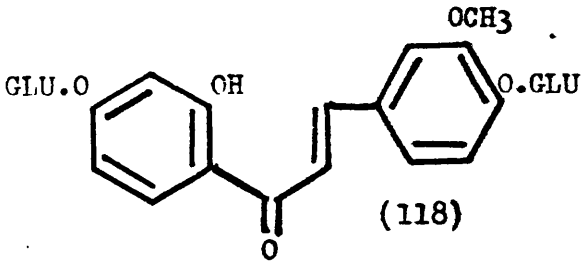


(116)

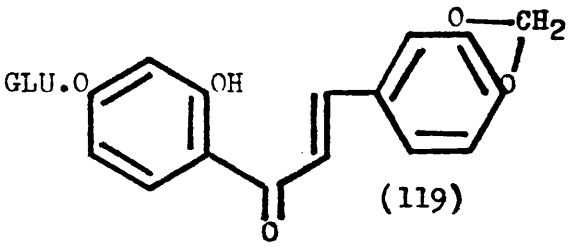


(117)

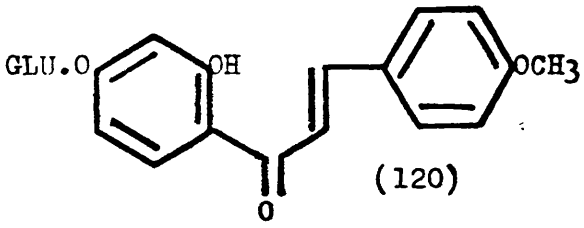
Reichel[66] has prepared several chalcones. That is, 2', 3, 4' - trihydroxy - 4 - methoxychalcone - 3,4' - di - (1-O-β-D - glucopyranoside)(117); 2',4,4' - trihydroxy - 3 - methoxy - 4,4' - di(1-O-β- D - glucopyranoside(118); 2',4'- dihydroxy - 3,4 - methylenedioxychalcone 4',-(1-O-β-D- glucopyranoside)(119) and 2',4' - dihydroxy- 4 - methoxychalcone - 4' - (1-O-β- D - glucopyranoside)(120).



(118)

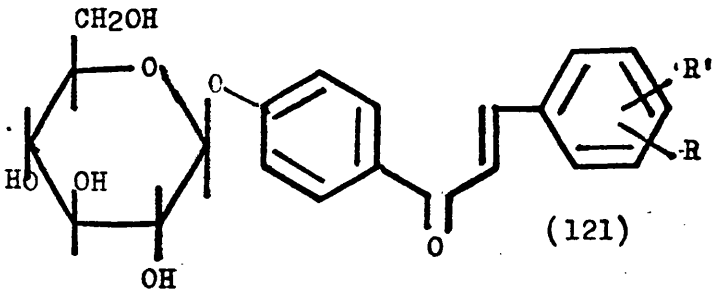


(119)



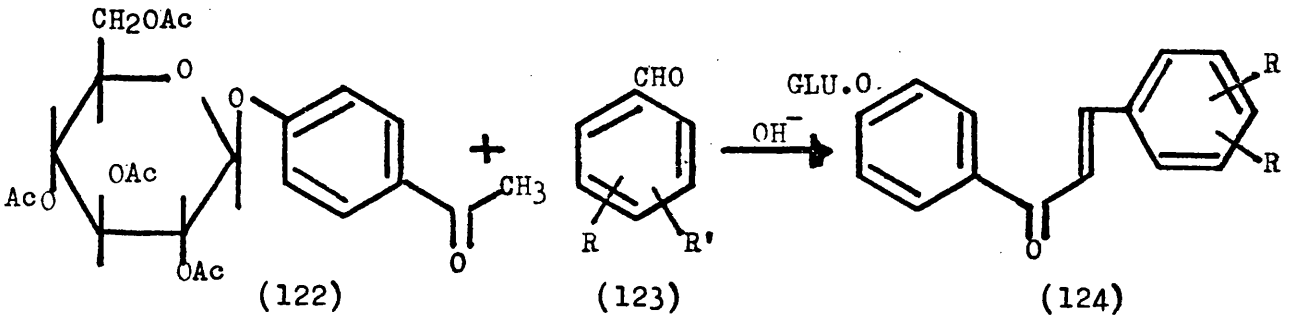
(120)

Chalcones glycosides have also been prepared (121) in which the 'A' ring is substituted only at the 4'- position with a glucosyloxy substituent.



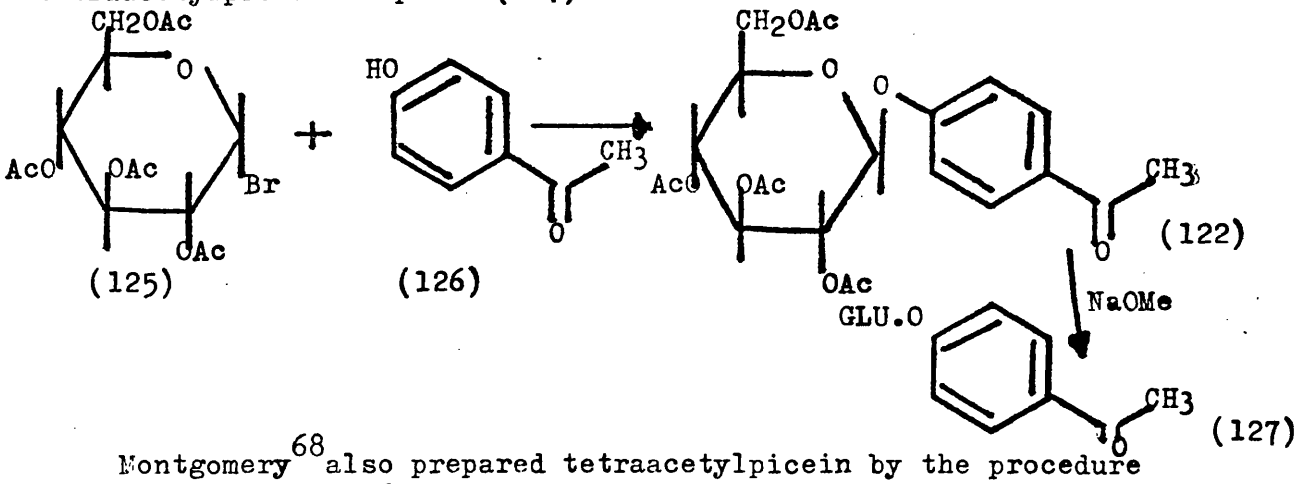
(121)

Most of these preparations were based on the reaction of tetra-acetylpicein(122) with a substituted aldehyde(123) to yield the chalcone glucoside(124) on treatment with alkali.



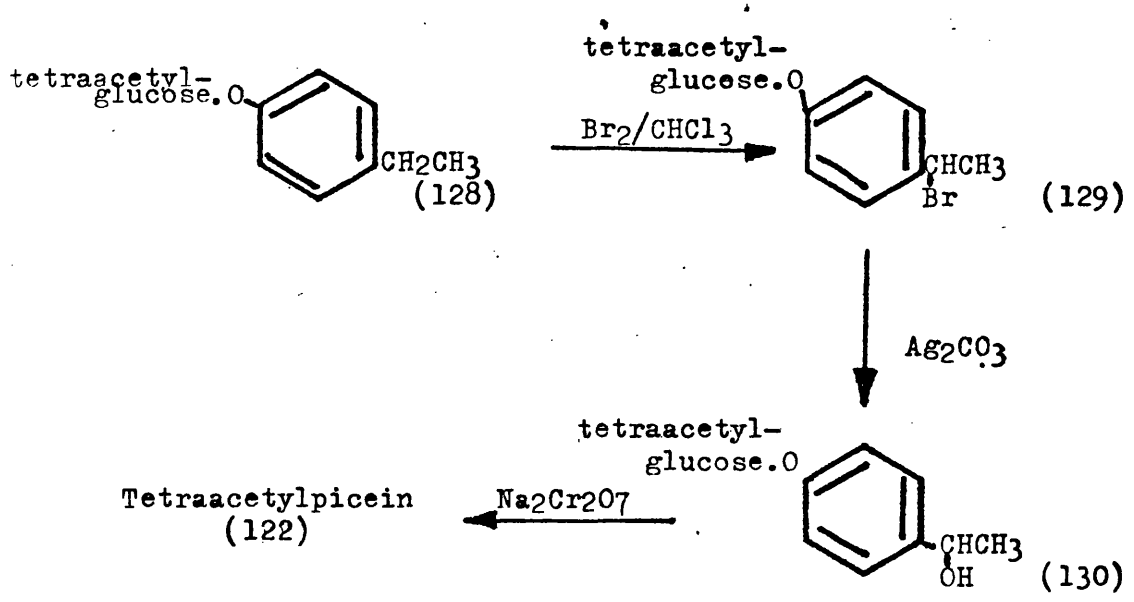
(122) (123) (124)

Tetraacetylpicein(122) was first synthesised by Mauthner[67] by adding an ethereal solution of α- acetobromoglucose(125) to a solution of 4-hydroxyacetophenone(126) in an aqueous solution of sodium hydroxide. The tetraacetylpicein(122) crystallised as colourless needles from methanol. Mauthner also deacetylated the tetraacetylpicein to picein(127).



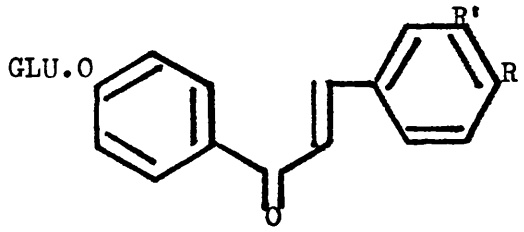
Montgomery[68] also prepared tetraacetylpicein by the procedure of Glaser and Wulwek[69] in a 75% yield and also deacetylated it to picein. Tanret[70] has isolated naturally occurring picein.

Helferich[71] has synthesised tetraacetylpicein by a different route by brominating 4-hydroxyethylbenzene - 4 - (1-O-β - D - tetraacetyl-glucopyranose) (128) to its bromo-derivative (129), followed by hydrolysis to the alcohol(130) and oxidation to tetraacetylpicein(122).



Only one reference has been found concerning the formation of chalcones from tetraacetylpicein and this is the paper of Bargellini and Leone[72] who prepared tetraacetylpicein by the method of Mauthner,

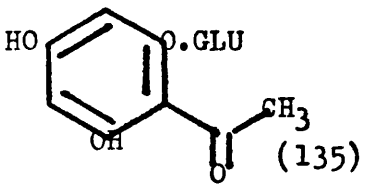
deacetylated this to picein and condensed the picein with substituted benzaldehydes to form 4' - hydroxychalcone - 4' - (1-O-β-D-glucopyranoside) (131); 4,4' - dihydroxy - 3 - methoxychalcone - 4' - (1-O-β-D- glucopyranoside) (132); 4' - hydroxy - 4 - methoxychalcone - 4' - (1-O-β-D- glucopyranoside) (133) and 4' - hydroxy - 3,4 - methylenedioxychalcone - 4' - (1-O-β-D - glucopyranoside (134).



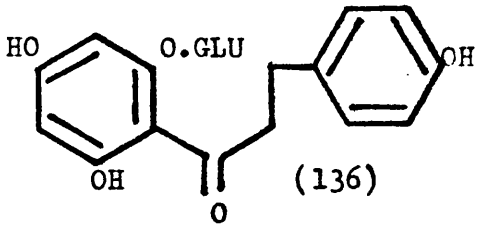
	R	R'
(131)	H	H
(132)	OH	OCH_3
(133)	OCH_3	H
(134)	$-OCH_2O-$	

The synthesis of chalcones and dihydrochalcones in which the 'A' ring possesses a phloroglucinol-type structure has been more extensively investigated.

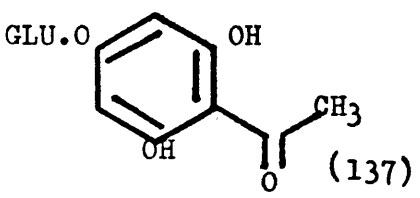
Zemplen and Bognar[73] prepared ortho-phloridzin (136) by reacting phloroacetophenone - 2 - (1-O-β - D - glucopyranose (135) with 4 - hydroxybenzaldehyde in strong alkali to form the chalcone followed by hydrogenation to the dihydrochalcone, ortho-phloridzin (136). Similarly, they prepared para-phloridzin (138) from, phloroacetophenone - 4 - (1-O-β - D - glucopyranose and 4 - hydroxybenzaldehyde.



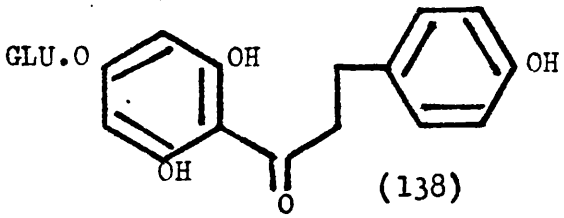
(135)



(136)

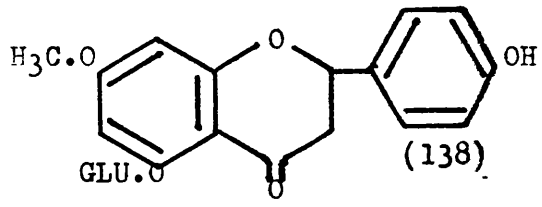


(137)

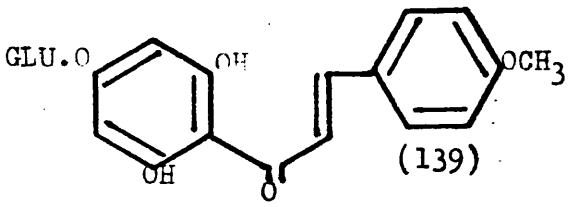


(138)

Zemplen and Bognar also synthesised hesperetin dihydrochlcone glucoside(90)[74], sakuranin (138) and isosakuranin (139)[75,76]. The sakuranin was converted to its dihydrochalcone, also.



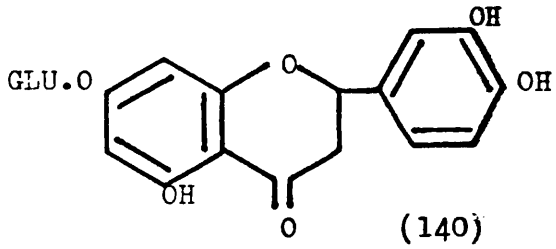
(138)



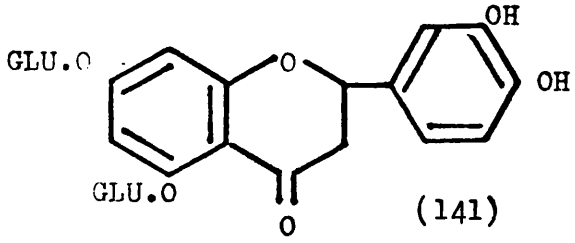
(139)

Bognar et al.[77] prepared mono- and diglucosides of phloroacetophenone and converted these to chalcone, flavanone and phloridzin-type glycosides.

Eriodictyol-7-glucoside(140) and its chalcone have been prepared[78,79] but Horhammer has also prepared a diglycoside(141).



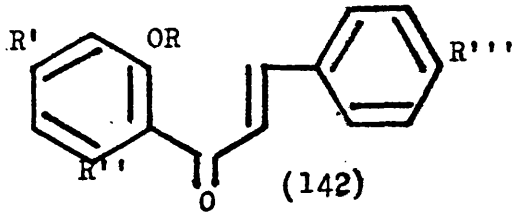
(140)



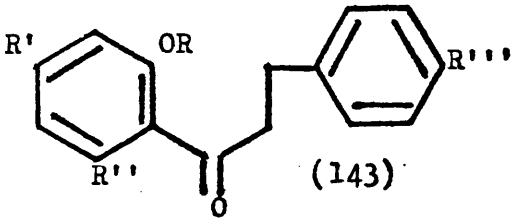
(141)

A series of chalcones and dihydrochalcones have been synthesised by Diedrich[80] by formation of various hydroxyacetophenone-glycosides with the glycoside substituted at the 2-position of the hydroxyacetophenone compound. These glycosides were prepared by reaction of the appropriate O-acetylglycosyl halide with the appropriate phenol in alkaline acetone. The chalcones(142) were formed by a Claisen-Schmidt-type condensation and then hydrogenated to dihydrochalcones(143). Both series of compounds prepared are shown in Table 6

Table 6 . Chalcones and dihydrochalcones prepared by Diedrich.



(142)

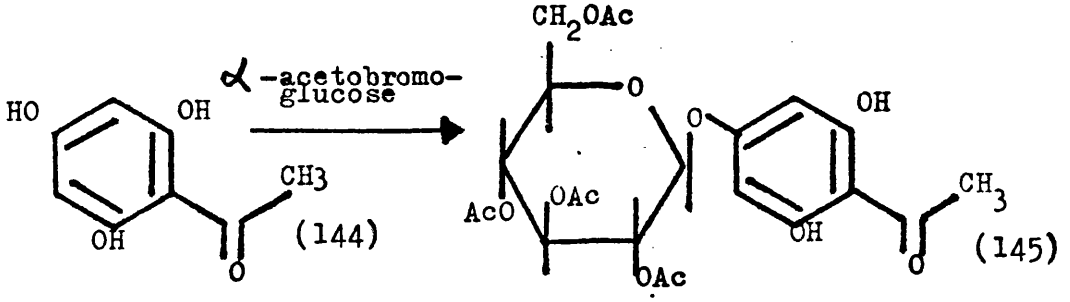


(143)

	R	R'	R"	R"'
1	galactose	-OH	-OH	-OH
2	2,4,6-tri-acetyl-3-methylglucose	-OH	-OH	-OH
3	glucose	-H	-OH	-OH
4	glucose	-OH	-OH	$-OCH_3$
5	glucose	-OH	-H	-OH

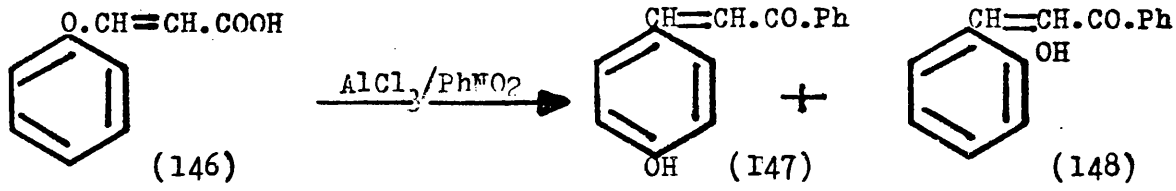
These compounds are of the phloridzin - type but are not 4'-glycosyloxydihydrochalcones, the compounds which are of most interest as potential sweeteners. It is also of interest to note that phloridzin inhibits the intestinal and renal absorbtion of glucose and other monosaccharides[81] the initial step being the orientation and/or binding of sugar onto a site on the membrane[82].

Phloroacetophenone - 4' - (1 - O -β- D - tetraacetylglucopyranoside) (145), the starting material for chalcone 4' - glucoside synthesis, has been prepared by Baker[83] in a 7.3% yield by reacting phloroacetophenone (144) and α-acetobromoglucose(125) in acetone/10% KOH in water. The mixture was shaken for two days, then poured onto ice, extracted with chloroform and the oil crystallised from methanol. This compound has also been prepared by Zemplen and Bognar[84] in yields of 10.3 and 11.1%.



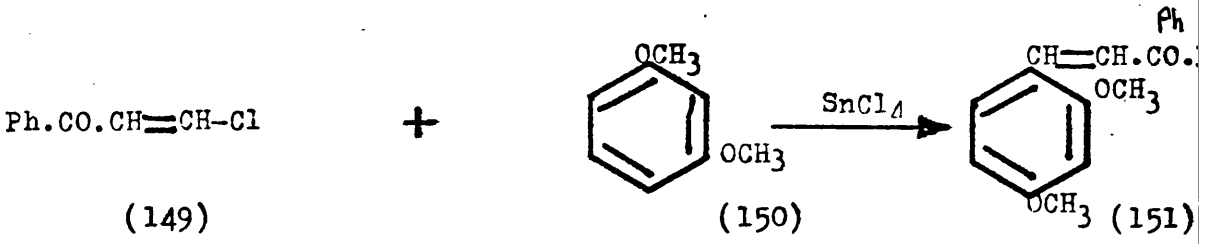
Some further examples of the synthesis of chalcone aglycones are described below. Simpson[85] has prepared chalcones by using a

Fries rearrangement of an $\alpha\beta$- unsaturated -β- aryloxy acid(146) to the chalcones(147 and 148).



(146) (147) (148)

Belyaev[86] has prepared chalcones(151) by reaction of β- chlorovinyl ketones(149) with alkoxy derivatives of benzene(150)



(149) (150) (151)

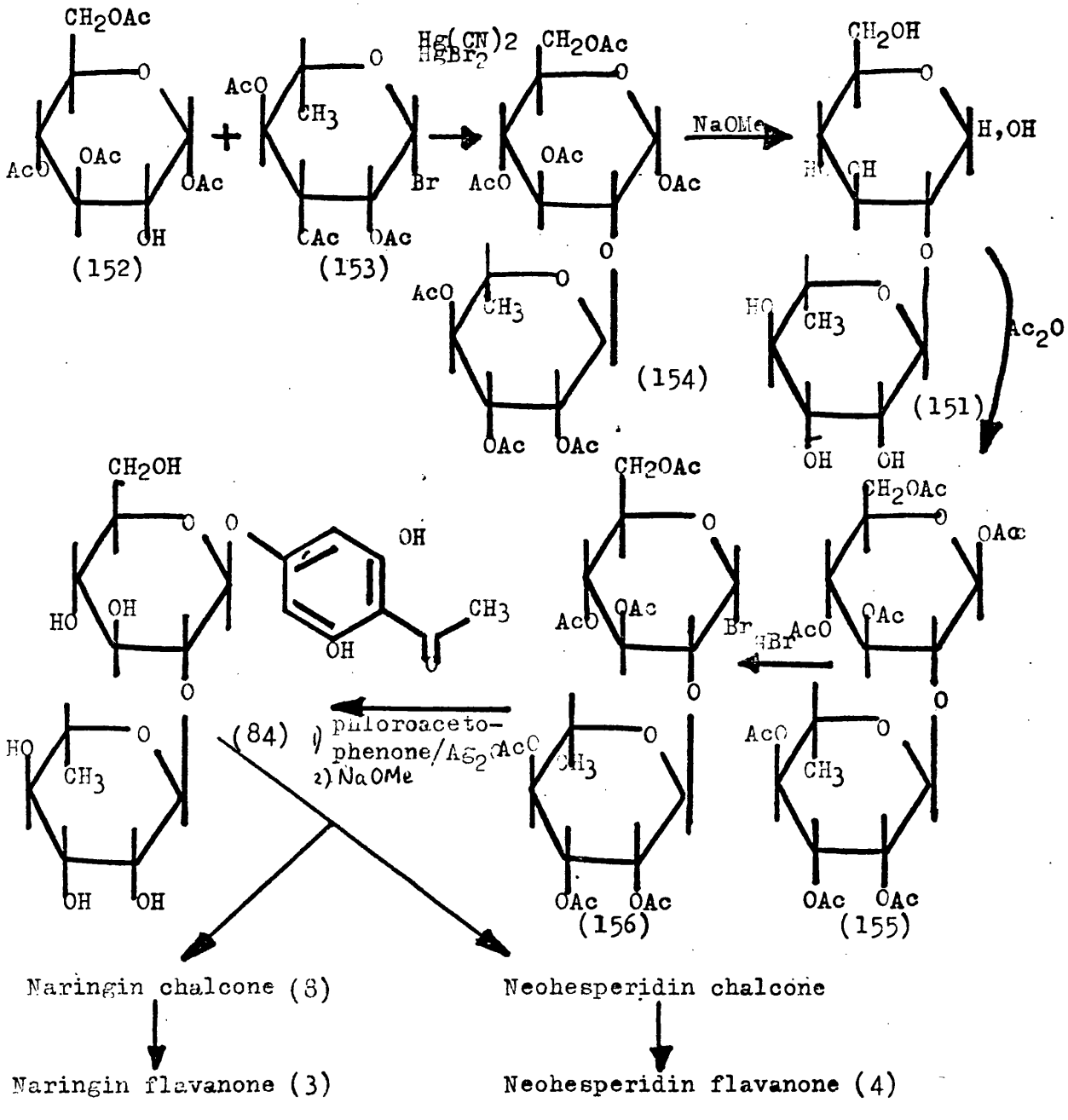
Langlais et al[87] have synthesised $\alpha\beta$- ethylenic ketones by reaction of organo-cadmium derivatives with $\alpha\beta$- unsaturated chlorides.

The Synthesis of Dihydrochalcone Disaccharides a) From neohesperidose

There are no references to the total synthesis of dihydrochalcone dissacharides in the literature but Kamiya[88] et al have synthesised naringin and neohesperidin chalcones from glucose, rhamnose, phloro-acetophenone and either 4 - hydroxybenzaldehyde or isovanillin.

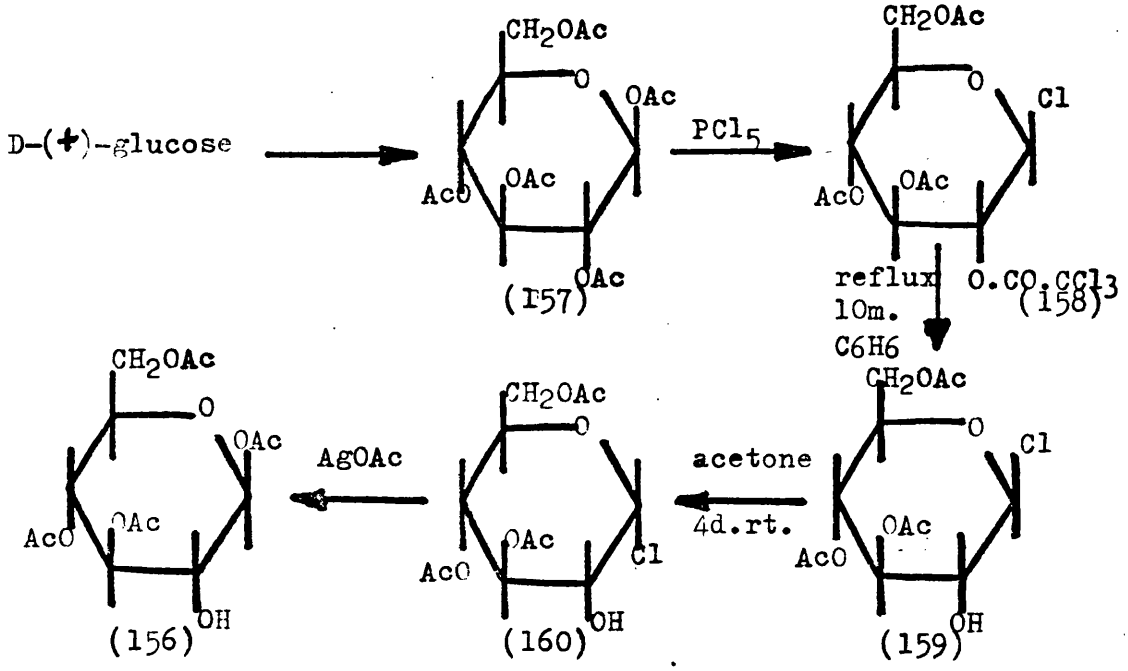
This group of workers investigated several methods for the synthesis of α- L - rhamnosyl disaccharides,[89] namely; the Königs - Knorr reaction by the use of a silver salt in chloroform solution; the Zemplen reaction by the use of mercuric acetate in benzene solution; the Helferich reaction by the use of a mixture of mercuric cyanide and mercuric bromide in acetonitrile solution and the Brederick reaction by the use of silver perchlorate in nitromethane solution. Neohesperidose (151) was best synthesised using the Helferich reaction and a condensation of α- 1,3,4,6 - tetra - O - acetyl - D - gluco-pyranose(152) and α- acetobromorhamnose(153) was accomplished. As the syrup containing the heptaacetyl compound(154) failed to crystallis it was deacetylated and chromatographed on a carbon/celite column. The disaccharide fractions were collected and on removal of the solvent an amorphous powder was obtained in 77% yield. Heptaacetylneohesperidose (155) was obtained via acetylation of the neohesperidose and this was

converted to hexaacetyl -α- neohesperidosyl bromide (156) by reaction with a hydrogen bromide solution in acetic acid. These authors were unable to obtain crystalline hexaacetyl -α- neohesperidosyl bromide. Phloroacetophenone and hexaacetyl -α- neohesperidosyl bromide(156) were reacted together using silver oxide and quinoline catalyst to yield a syrup which was chromatographed on a cellulose column and eluted with the solvent -n- butanol/acetic acid/water 4:1:1. The fraction containing phloroacetophenone - 4' -β- neohesperidoside(84) was collected to yield, finally, the product as fine needles. Condensation of compound (84) with isovanillin yielded neohesperidin (4) via its chalcone and neohesperidin flavanone and condensation with 4 - hydroxybenzaldehyde yielded naringin flavanone (3) via its chalcone (8).



Neohesperidose has also been synthesised by Koeppen[90]. He draws attention to the fact that reported syntheses[89,91] have involved the condensation of 1,3,4,6 - tetra - O - acetyl -α - D - glucopyranose (152) with α- acetobromo-rhamnose (153) to form a non-crystalline α- neohesperidose derivative (154) which was deacetylated and then acetylated to give the crystalline β- heptaacetate of neohesperidose (155). Koeppen's paper describes the synthesis of this same β - heptaacetate in 67% yield by reaction of the anomeric 1,3,4,6 - tetra - O - acetyl -β - D - glucopyranose (156) with α- acetobromorhamnose. In contrast, the yield obtained by Kamiya for this same compound was 16%.

However, whereas 1,3,4,6 - tetra - O - acetyl -α - D - glucopyranose is readily prepared directly from glucose by the method of Helferich and Zirner[92] (that is by the hydrolysis of α- acetobromoglucose in an aqueous sodium acetate solution), 1,3,4,6 - tetra - O - acetyl -β - D - glucopyranose (156) is only prepared by a five-stage synthesis from glucose via the pentaacetate (157), 3,4,6 - tri - O - acetyl - 2 - O - trichloroacetyl -β - D - glucopyranosyl chloride (158), 3,4,6 - tri - O - acetyl -β - D - glucopyranosyl chloride (159) and 3,4,6 - tri - O - acetyl - α- D - glucopyranosyl chloride (160)

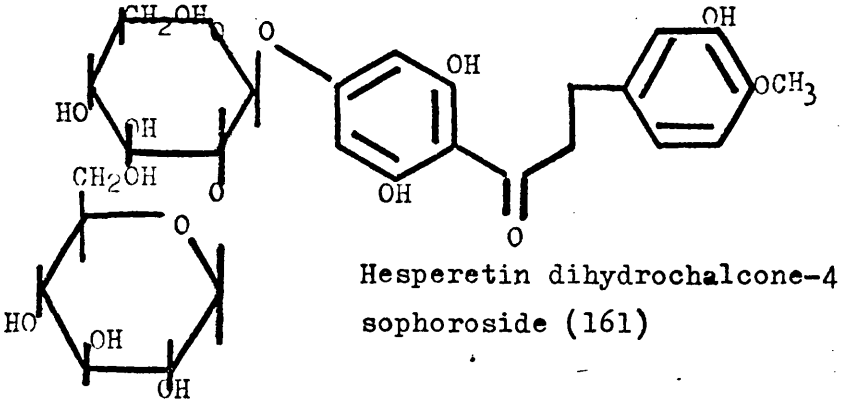


Koeppen used the method of Lemieux and Howard[93] to prepare compounds (158)and(159). The formation of compound(160)is described by Lemieux and Huber[94]. Although with the exception of the trichloroacetyl derivative, all the intermediates can be prepared in excellent yield,

the overall yield from glucose was at best only 10%. Koeppen therefore sought an alternative method for the preparation of β - neohesperidose heptaacetate and accomplished this by condensing 1,3,4,6 - tetra - O - acetyl -α- D - glucopyranose (152) with α- aceto - bromorhamnose (153) and converting the resultant α - neohesperidose heptaacetate (154) to the β- anomer (155) via the acetylated glycosyl bromide (156). The β- neohesperidose heptaacetate was thus prepared in a yield of 63% from compound (152) and pure neohesperidose was crystallised from solution as the β- monohydrate.

b) Dihydrochalcones with a 2 - O - glycosylglucopyranose moiety.

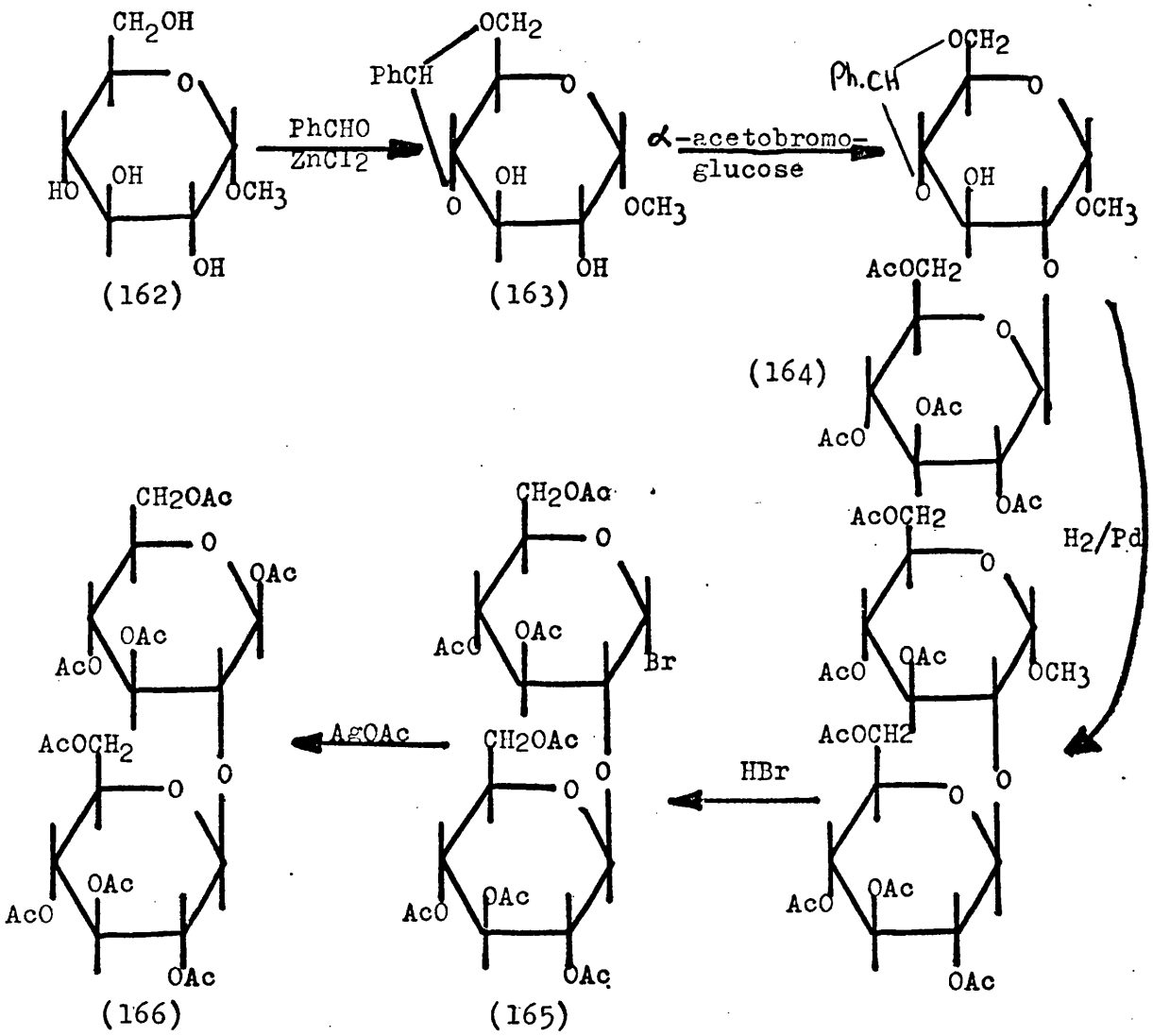
Examples of the synthesis of dihydrochalcones with a 2 - O - glycosylglucopyranose moiety have not been reported in the literature. Hesperetin dihydrochalcone-4'-β-sophoroside (161) might be prepared by condensation of the α- bromoacetylsophorose (165) with phloroacetophenone and formation of the dihydrochalcone via the chalcone in the usual way.



Hesperetin dihydrochalcone-4'-β-sophoroside (161)

The feature which is common to the syntheses of disaccharides is that a glycoside is first prepared with almost all its hydroxyl groups blocked, then the disaccharide link is formed by reaction with a bromo-saccharide. The synthesis of oligosaccharides has been reviewed by Evans[95] whilst the determination of the configuration of glycosidic linkages in oligosaccharides has been reviewed by Charlson[96]. A review concerning glycoside synthesis has appeared by Conchie.[97] In the case of 2 - O - glycosylglucopyranoses it is necessary to block the hydroxyl groups on the 1-,3-,4 - and 6 - positions. The formation of 1,3,4,6-tetraacetyl-β-D-glucopyranose and its α- analogue have already been described (see page 39) in respect of their combination with α- acetobromorhamnose to form neohesperidose (2 - O -α- L - rhamnopyranosyl - D - glucopyranose). It is possible to synthesise sophorose (2 - O -β- D - glucopyranosyl - D - glucopyranose) by a similar condensation between 1,3,4,6 - tetraacetyl -β- D - glucopyranose

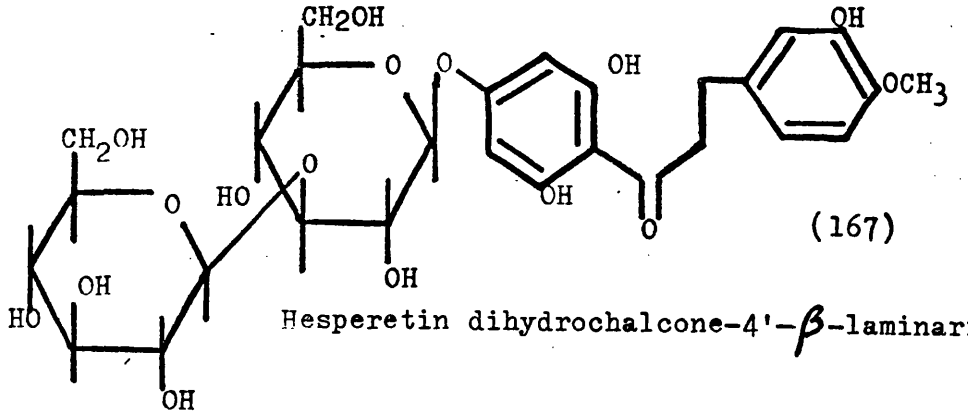
and its α- analogue and α- acetobromoglucose. This has been carried out by Gakhokidze[98] who also reacted 2,3,4,6 - tetraacetyl - D - glucopyranose with 1,3,4,6 - tetraacetyl - D - glucopyranose with zinc chloride catalyst to form the same disaccharide. Schmidt[99] has also prepared sophorose but his method was to prepare methyl α - D - glucopyranoside(162) then methyl - α - D - (4,6 - benzylidene-glucopyranoside)(163) and to condense this with α - acetobromoglucose with silver carbonate catalyst to form the disaccharide compound (164). The benzylidene blocking group was removed by hydrogenation and the methyl group was displaced by hydrogen bromide to form the α - bromide of sophorose (165) and finally the sophorose octaacetate(166).



The α-anomer of sophorose is kojibiose (2-O-α-D-glucopyranosyl-D-glucopyranose) and this latter compound is not the preferred and stable form—the β-anomer, sophorose is the stabler. Work on kojibiose has been reported[100].

6) Dihydrochalcones with a 3-O-glycosylglucopyranose moiety.

There is no reported synthesis of a dihydrochalcone with a 3-O-glycosylglucopyranose moiety. Hesperetin dihydrochalcone-4'-β-laminaribioside(167) might be prepared by condensation of α-acetobromolaminaribiose with phloroacetophenone and formation of the dihydrochalcone via the chalcone.

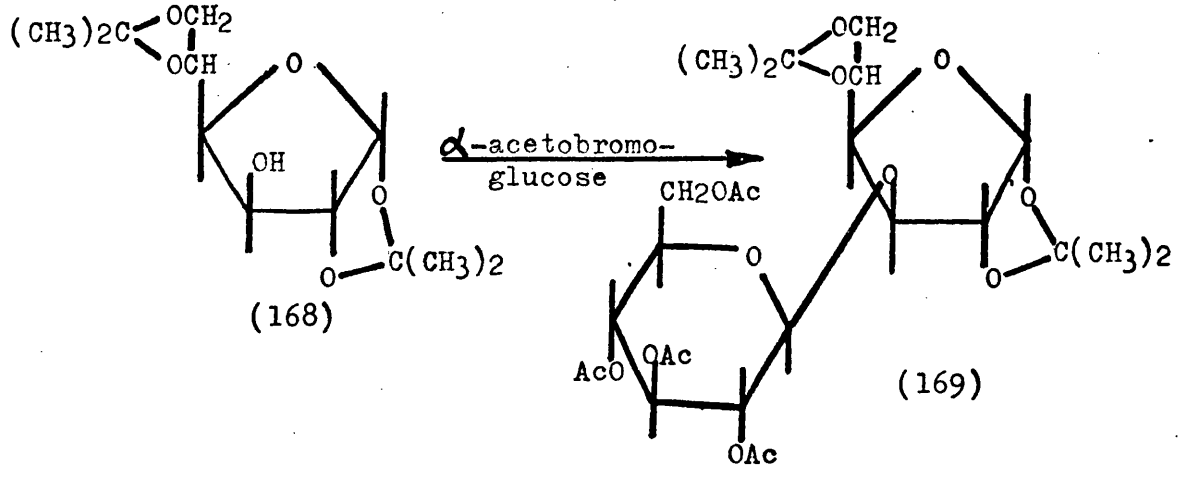


(167)

Hesperetin dihydrochalcone-4'-β-laminaribioside

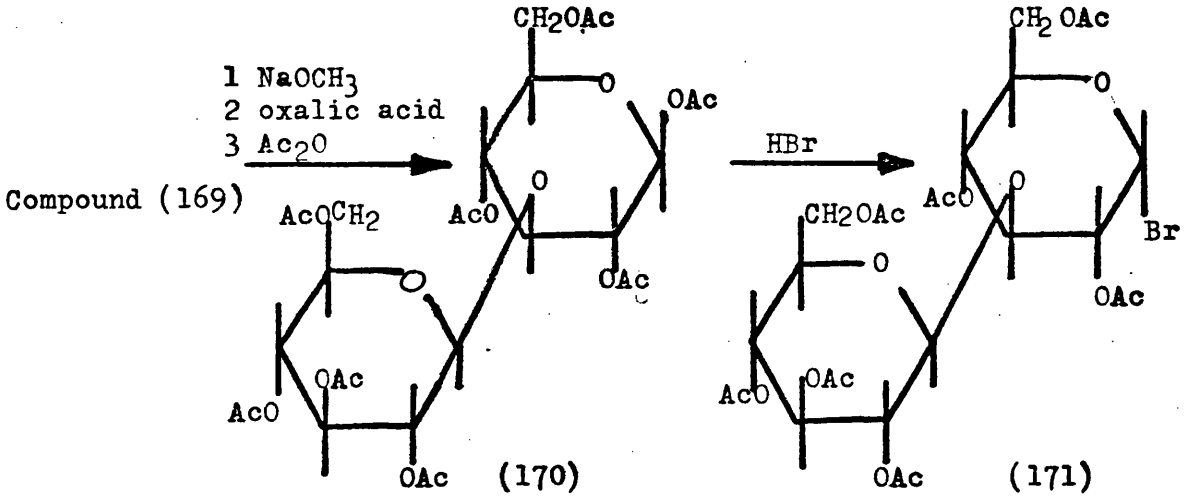
Laminaribiose(3-O-β-D-glucopyranosyl-D-glucopyranose) can be prepared from the polysaccharide laminarin[101,102]. This disaccharide has also been prepared by making use of 1,2-5,6-di-O-isopropylidene-D-glucofuranose(168) in which only the 3-hydroxyl group is free. Thus, Freudenberg[103,104] condensed α-acetobromoglucose with compound (168) to form the substituted laminaribiose compound(169) which was deacetylated by boiling in sodium methoxide solution, treating with oxalic acid to remove the isopropylidene groups and then reacetylating the mixture to form octaacetyllaminaribiose(170) and finally α-acetobromolaminaribiose(171).

Bachli and Percival[102] found that a sample of synthetically prepared laminaribiose was identical to a sample obtained from laminarin.

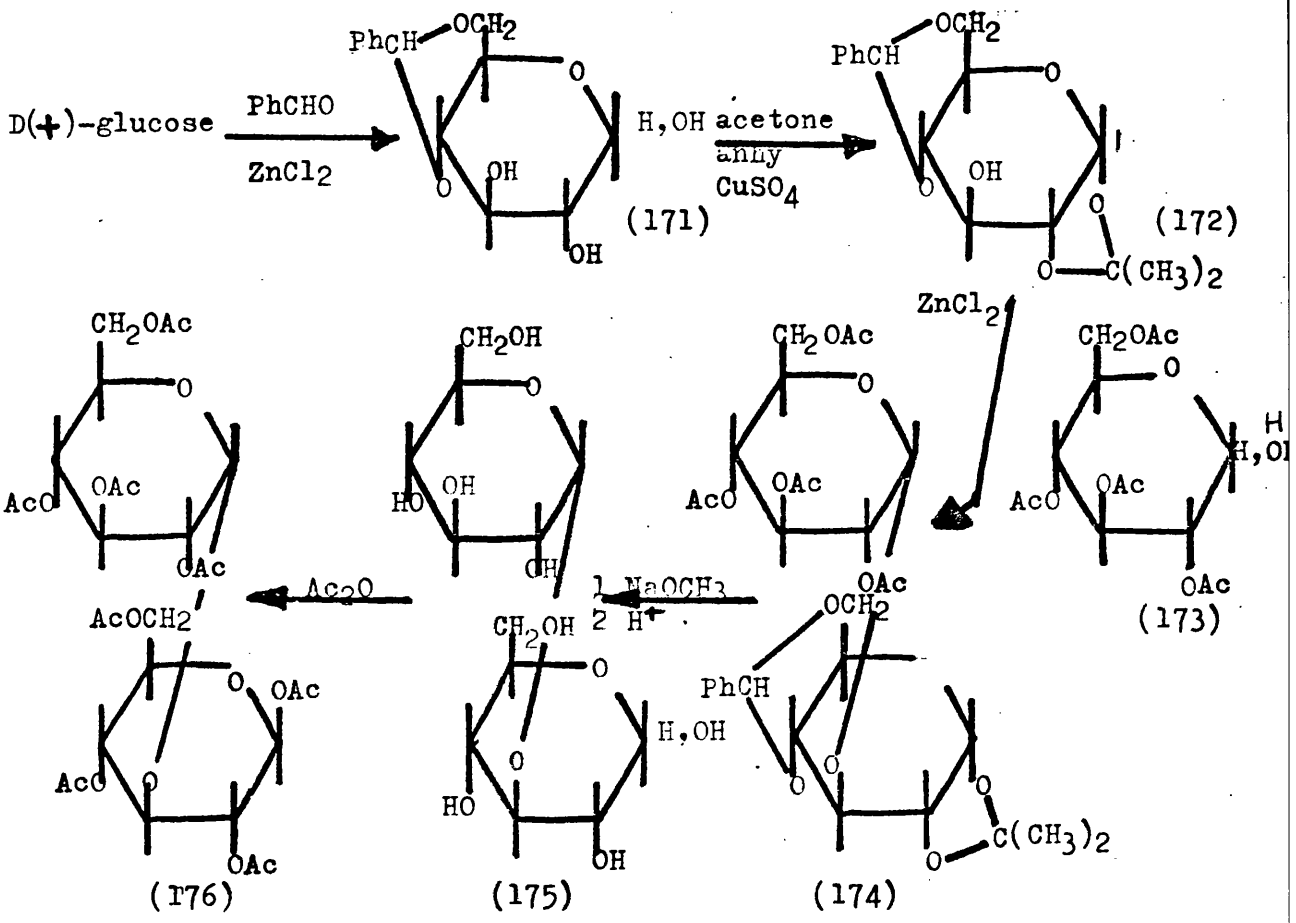


(168)

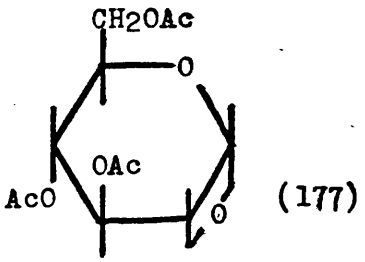
(169)



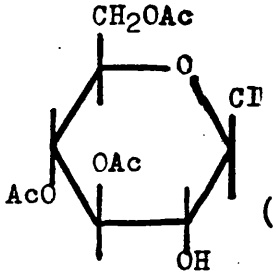
The α-anomer of laminaribiose is nigerose or sakebiose (3-O-α-D-glucopyranosyl-D-glucopyranose) and its synthesis was first achieved by Gakhokidze[105].He prepared 4,6-O-benzylidene-D-glucopyranose (171), then 1,2-O-isopropylidene-4,6-O-benzylidene-D-glucopyranose(172) which he condensed with 2,3,4,6-tetra-O-acetyl-D-glucopyranose(173) using zinc chloride catalyst.The resultant disaccharide(174) was treated with hot sodium methoxide solution to remove the acetyl groups and boiled with dilute sulphuric acid to remove the benzylidene and isopropylidene blocking groups to yield nigerose(175).This was acetylated to nigerose octaacetate(176).



It is not easy to prepare α-glucosydic disaccharides, however, the β-form is usually the more stable anomer and therefore the one more easily prepared. Brigl's anhydride (3,4,6-tri-O-acetyl-1,2-anhydro-α-D-glucopyranose)(177)[106] has been used to achieve the synthesis of α-disaccharides in which the linkage occurred at the 1- or 2- positions. For α-disaccharide synthesis a tetra-O-acetyl-β-glucopyranosyl halide is required but β-halides are generally unstable, the α-halide being the preferred form. 3,4,6-tri-O-acetyl-1-chloro-β-D-glucopyranose (178)[107] is reasonably stable and would be chosen in preference to Brigl's anhydride.



(177)

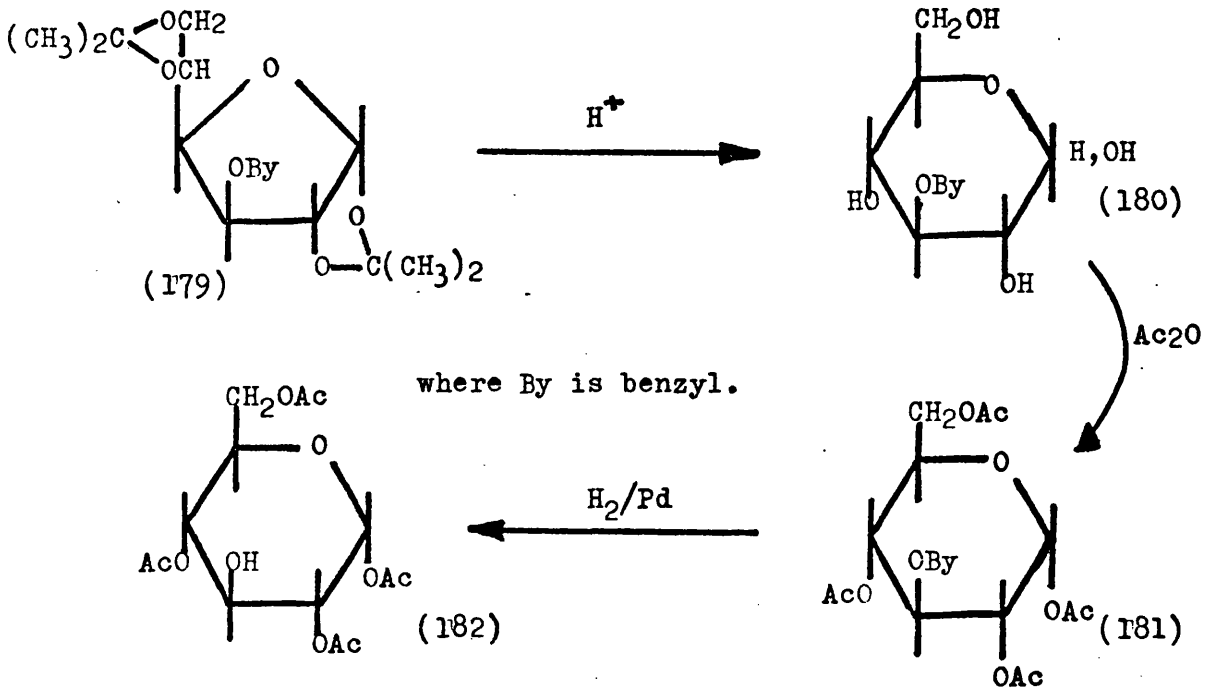


(178)

Thus, Gilbert and Smith[108] refluxed 1,2-4,6-di-O-isopropylidene-D-glucofuranose(168) and 3,4,6-tri-O-acetyl-1-chloro-β-D-glucopyranose (178) in toluene. Only 1.8% of pure disaccharide was obtained and it was found impossible to preferentially hydrolyse the isopropylidene residues without severing the inter-sugar linkage.

Barker[109] obtained nigerose from the partial hydrolysate of the fungal polysaccharide nigeran and Haq and Whelan[110] have also carried out a chemical synthesis.

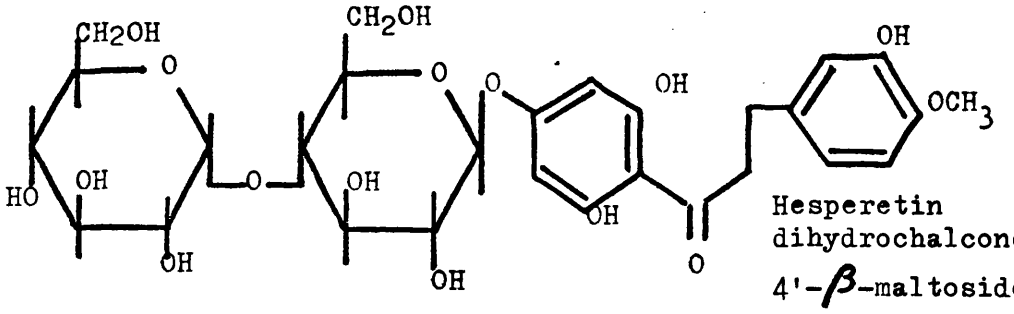
Another possible route to 3-O-glycosylglucopyranose disaccharides which has not been reported, apparently, would be to prepare 1,2-4,6-di-O-isopropylidene glucofuranose[111], to benzylate the free 3-hydroxyl group of this compound to form 1,2-4,6-di-O-isopropylidene-3-benzyloxy-D-glucofuranose(179)[112]. Acid hydrolysis of compound (179) yields 3-benzyloxy-D-glucopyranose (180)[112] which on acetylation yields 1,2,4,6-tetra-O-acetyl-3-benzyloxy-α-D-glucopyranose(181)[113] and finally, on hydrogenation yields the useful intermediate 1,2,4,6-tetra-O-acetyl-α-D-glucopyranose(182)[113]. This intermediate compound(182) would make a useful starting intermediate for disaccharide synthesis.



where By is benzyl.

d).Dihydrochalcones with a 4-O-glycosylglucopyranose moiety.

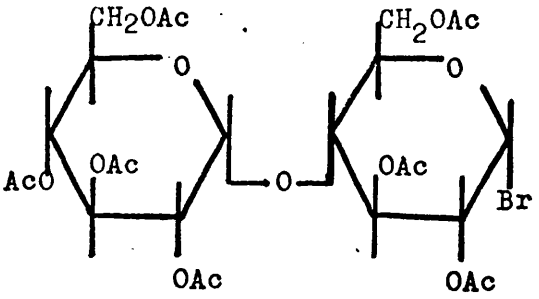
There is no reported synthesis of a dihydrochalcone with a 4-O-glycosylglucopyranose moiety.Hesperetin dihydrochalcone-4'-β-maltoside (183)might be prepared,for example, by condensation of α-acetobromo-maltose with phloroacetophenone and formation of the dihydrochalcone via the chalcone.



Hesperetin dihydrochalcone-4'-β-maltoside(183)

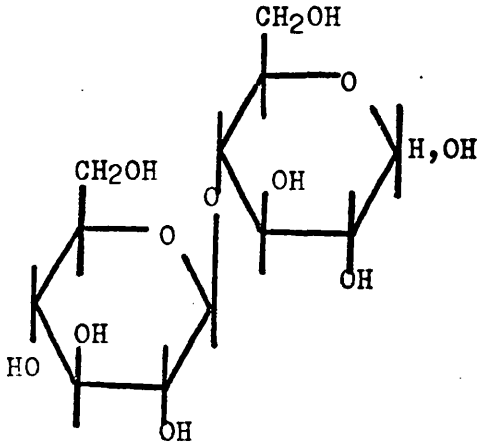
Maltose (4-O-α-D-glucopyranosyl-D-glucopyranose) is readily available commercially being obtained from starch by hydrolysis with β-amylase.

In order to prepare hepta-O-acetyl-α-maltosyl bromide (184) Brauns[114] and also Hudsen and Sayre[115] reacted maltose with hydrogen bromide in acetic acid. Fisher and Fisher[116] reacted maltose with acetyl bromide whilst Karrer and Nagel[117] used either acetyl bromide or methyl bromide.



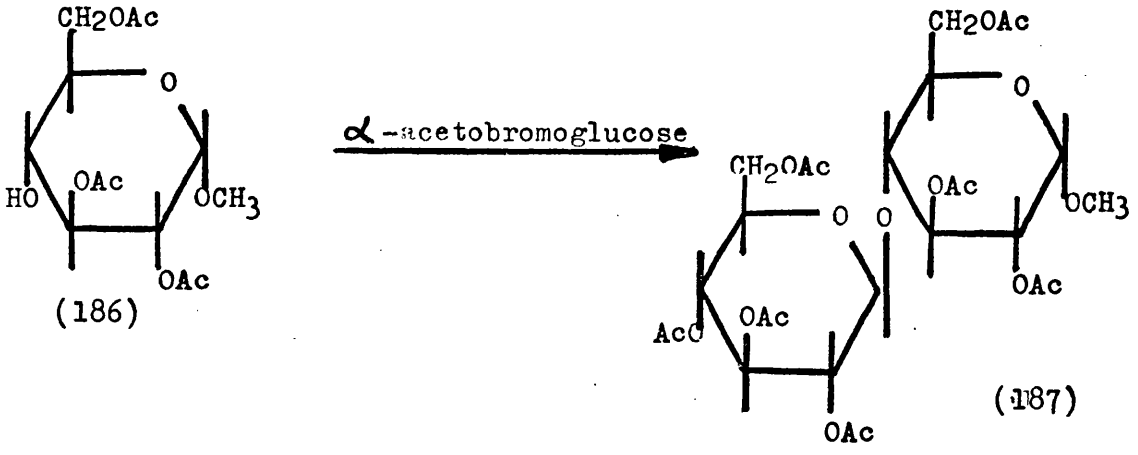
(184)

The β-anomer of maltose is cellobiose (4-O-β-D-glucopyranosyl-D-glucopyranose)(185)which is available commercially.



(185)

The synthesis of cellobiose has been achieved by Freudenberg and Nagei[118] who reacted 1,6-anhydro-β-D-glucopyranose with α-acetobromoglucose(101). Helferich and Brennen[119] also achieved a synthesis of methyl heptaacetylcellobioside(187) by reacting 2,3,6-tri-O-acetyl-β-D-(1-O-methylglucopyranose)(186) with α-acetobromoglucose(101).



(186)

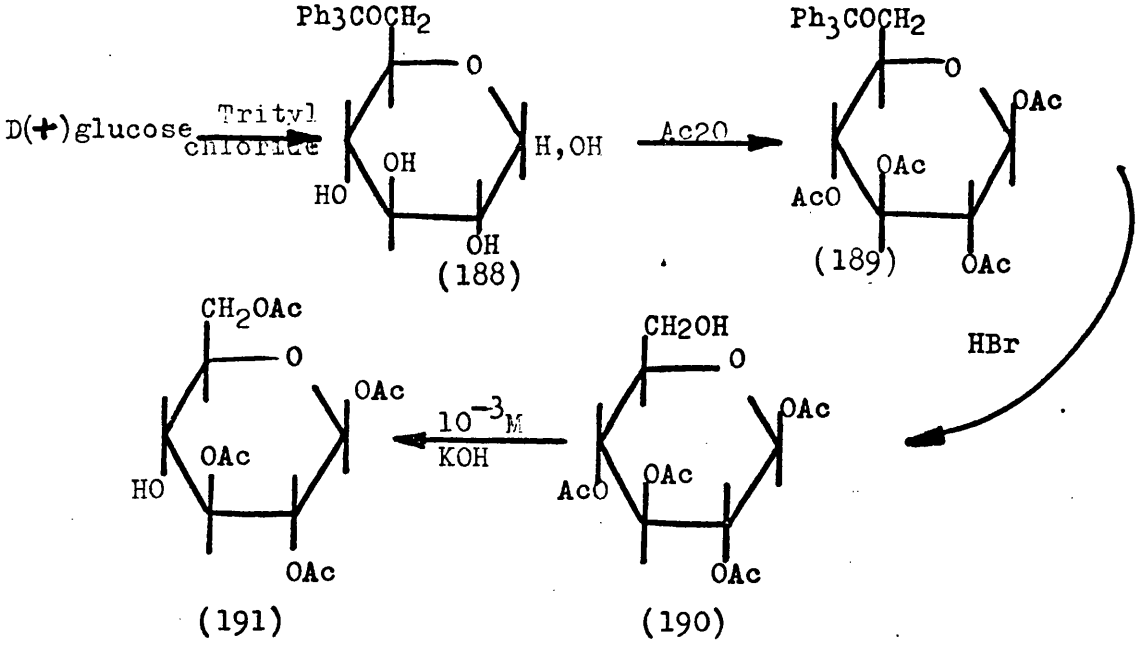
(187)

Gilbert[120] prepared 1,2,3,6-tetra-O-acetyl-β-D-glucopyranose from 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose by the acyl migration method of Helferich and Müller[121] and reacted the former compound with α-acetobromoglucose to form octa-O-acetylcellobiose which they were unable to crystallise.

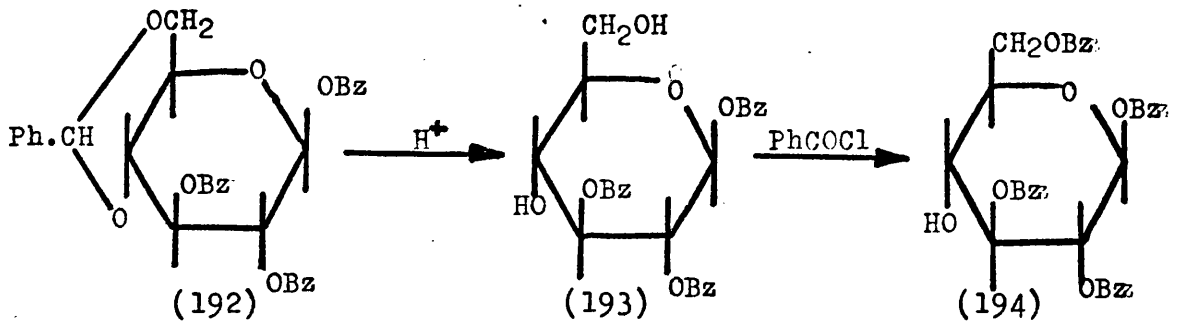
α-acetobromocellobiose has been prepared by Zemplen[122], Fisher and Zemplen[123] and also by Stevens and Blumberg[124] by reaction of hydrogen bromide in acetic acid with cellobiose.

Stanek and Kokourek[125] have prepared phenyl glycosides of cellobiose by reacting α-D-cellobiosyl bromide with several phenols in a solution of sodium hydroxide in aqueous acetone.

In order to prepare 4-O-glycosylglucopyranose disaccharides it is of interest to prepare derivatives in which all but one of the hydroxyl groups are protected. One such derivative is 1,2,3,6-tetra-O-acetyl-β-D-glucopyranose(191) which can be prepared from D-glucose by first making 6-O-trityl-D-glucopyranose(188) by reaction with trityl chloride[126], acetylating this compound to 1,2,3,4-tetra-O-acetyl-6-O-trityl-β-D-glucopyranose(189)[126,127,128] then removing the trityl group with hydrogen bromide in acetic acid solution to form 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose(190)[127,128,129]. In 10^{-3}M potassium hydroxide solution the 4-acetyl group of compound (190) migrates to the 6-position to form 1,2,3,6-tetra-O-acetyl-β-D-glucopyranose(191)[128,129,130].



An alternative intermediate which has all but its 4-hydroxyl group benzoylated is 1,2,3,6-tetra-O-benzoyl-β-D-glucopyranose(194). This compound can be prepared by forming 4,6-O-benzylidene-D-glucopyranose(171) and benzoylating this compound with benzoyl chloride to 1,2,3-tri-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranose(192)[131]. The benzylidene group is removed by acid hydrolysis to form 1,2,3-tri-O-benzoyl-β-D-glucopyranose(193)[131] which is benzoylated at the



6-position by a room-temperature treatment with benzoyl chloride to form 1,2,3,6-tetra-O-benzoyl-β-D-glucopyranose(194)[131,132].

5) Sweetness and Structure

The structure of several dihydrochalcones is shown in Table 2 on page 5.An examination of the nature of the structure of those which are sweet reveals the following facts:

1) Naringin DHC is sweet whereas hesperidin DHC is tasteless. Only the dihydrochalcones which possess a neohesperidose or glucose moiety are sweet,those which possess a rutinose moiety are not.

2) Of the neohesperidosyl dihydrochalcones which are substituted in the "B" ring with hydroxy groups,the meta-(14) and para-monosubstituted (9) compounds are sweet whilst the ortho-substituted compound(13) is bitter.Also,the 3,4-disubstituted hydroxy compound (15) is slightly sweet and the 3,4,5-trihydroxy compound (19) has no sweetness.

3) The sweetest neohesperidosyl dihydrochalcones are hydroxy/alkoxy disubstituted dihydrochalcones.Thus,naringin DHC (9),neohesperidin DHC (10) and the ethoxy and n-propoxy analogues of neohesperidin DHC (17 &18) are intensely sweet.The 2-hydroxy-3-methoxy analogue is also sweet but unexpectedly,the effect of reversing the hydroxy and alkoxy substituents of neohesperidin DHC (10) and its ethoxy analogue (17) to form the 3-methoxy-4-hydroxy analogue (26) and the 3-ethoxy-4-hydroxy analogue (22) was to produce two tasteless compounds.

4) One might have expected the 3,5-dihydroxy-4-methoxy analogue to be sweet because of its obvious relation to neohesperidin DHC (10). It exhibits no sweetness,however,neither does the 2,4-dimethoxy-3-hydroxy analogue (24).

5) Of the dihydrochalcones which possess no hydroxy-substituents, poncirin DHC (16) and the 3,4-dimethoxy analogue (25) are mostly bitter, though they do have a trace of sweetness.The C-methyl compound,

that is the 3-methyl-4-methoxy analogue,(27) is also tasteless.

The conclusions which can be reached are as follows:

a) An hydroxyl group is necessary for sweetness(see compounds 9,10, 14,15,17,18,20.) but its presence does not guarantee it (see compounds 13,19,22,23,24,26.).

b) The absence of a hydroxyl group assures non-sweetness (see compound 27) or bitter sweetness (see compounds 16,25.).

c) For sweetness to subsist in hydroxy/alkoxy disubstituted compounds the order of the groups must be R-H-OH-alkoxy (see compounds 10, 17,18,20.) or R-OH-alkoxy (see compound 21).

d) Taste is abolished if the order of groups in hydroxy/alkoxy disubstituted compounds is R-H-alkoxy-OH (see 22,26).It is also abolished if three adjacent groups are present in addition to R (see 19,23,24.).

[133]

Horowitz has postulated that the receptor sites on the taste-cell membrane are capable of interacting with these dihydrochalcones.He assumes that,as a minimum,the receptor has sites which respond to 1) hydroxyl,2) alkoxyl and 3) the glycosidic and adjacent parts of the molecule (i.e. the whole molecule excluding the "B" ring.Horowitz concerned himself mainly with the part of the receptor which complexes with the hydroxy- and alkoxy-substituents of the "B" ring and assumes that the receptor allows free entry of the dihydrochalcone molecule but which limits the possible twisting and rotational orientation of the "B" ring.His ideas are shown diagramatically in Diagrams 1 to 4 below:

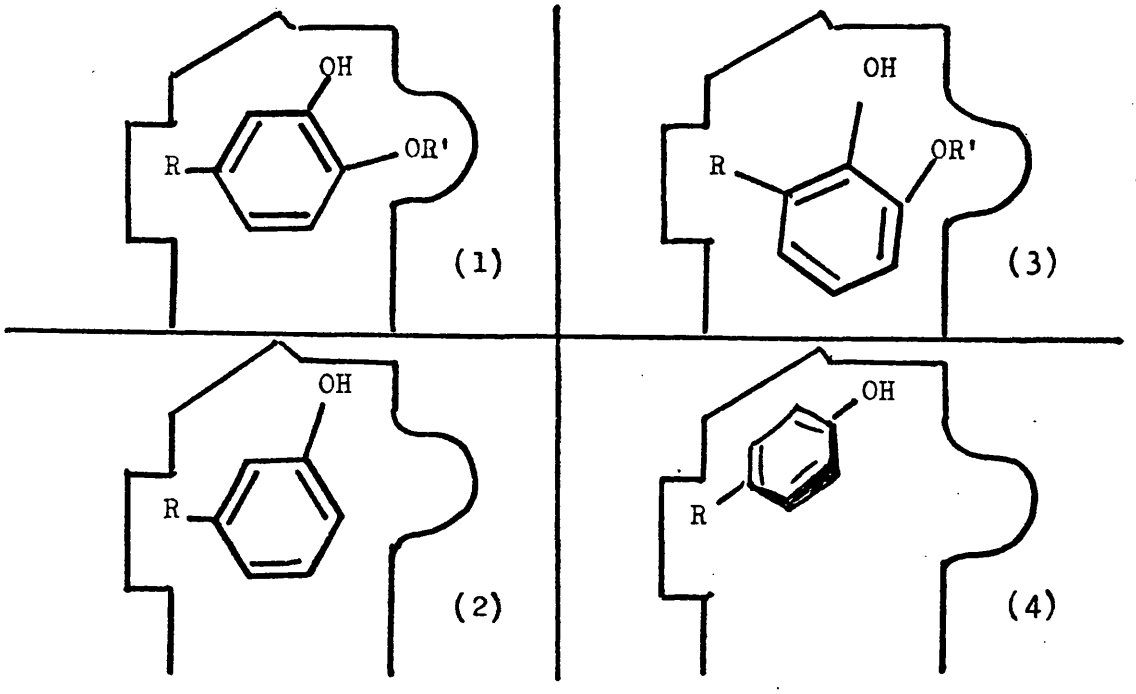


Diagram 1 represents the best fit-that of the 3-hydroxyl-4-alkoxy compounds.In the case of the 3-hydroxy compound,fewer receptor sites are bound with the result that this compound exhibits less sweetness than the type shown in Diagram 1. In Diagram 3 the "B" ring is twisted clockwise to provide the fit--the large R group is assumed to be flexible.Diagram 4 represents the complex with naringin DHC (9).To obtain the proper fit the "B" ring is twisted counterclockwise and secondly the ring is rotated out of the vertical plane along the ROH axis in order to minimise interference between the upper surface and the protons at the 2 and 3 positions. As far as non-sweet or bitter/sweet dihydrochalcones are concerned let us take compound(26) as an example. The presence of an alkoxyl group at position 3 or 5 of a molecule such as that shown in Diagram 4 would substantially reduce the partial rotation of the "B" ring because it is a more bulky group than the proton it has replaced.Therefore,it is likely that effective hydroxy/receptor complexing would be inhibited.A similar argument explains why the slightly sweet dihydroxy compound (15) and the tri-substituted derivatives (19,23&24) are unable to couple effectively to the receptor.

The nature of the receptor is not known but it is known that about fifty taste-cells occupy each taste-bud,that the sweet material which is producing an effect binds weakly to the receptor and that a receptor on one cell may produce a response of highly varying intensity when compared with a receptor on another cell.Indeed,different receptors on the same taste-cell give differing responses.The taste-cell is electrically charged and its potential changes depending upon whether or not it is in the process of stimulation.

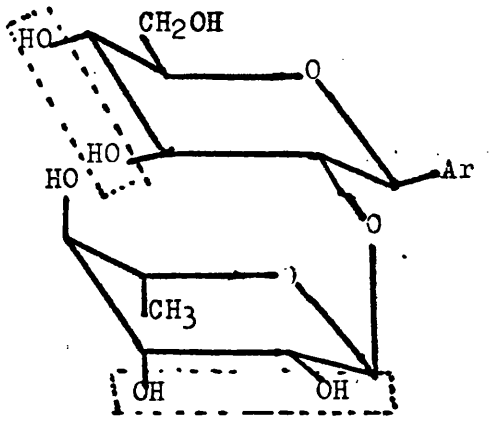
In the olfactory organ both nerve and receptor are part of the same cell but in the case of the taste-bud the receptor and nerve are separate.The nerve,therefore,is stimulated probably by a chemical mediator which originates in the receptor cell and which is transmitted across the cell junction or synapse to the nerve.It is depolarisation of the sense cell which leads to nerve stimulation.

The details of this process are unclear.How does the sweetener bind to the receptor?Why is binding necessary in the first place if the sweetener's effect is to depolarise the taste cell membrane? Is binding required so that the strongly polar substituents of sweeteners (the sulphonyl group of saccharin,for example) can exert their effect in mopping up electrons? Perhaps,there are no sweetness receptors at all.Perhaps,the sweetener has a gross effect on the taste cell and

depolarises the cell or a portion of it by a random transfer of charge from the cell to and/or from the sweetener? Anyway,it must be borne in mind that only water-soluble substances can be tasted and that no transport of sweeteners across the cell membrane occurs[134]. This latter fact is important because it could easily be postulated that the disaccharide moiety is simply an aid to transport and yet the rutinose compounds are not sweet and one might expect the transport properties of the neohesperidosyl and rutinosyl dihydrochalcones to be similar.

It is also of interest to remember that the cell membranes are heterogeneous and contain many varieties of receptor sites.The basic tastes of acid,sour and salty have to be tasted in addition to the sweet sensation and it seems that these different tastes are detected by the same taste cells,there is no taste cell specialisation.

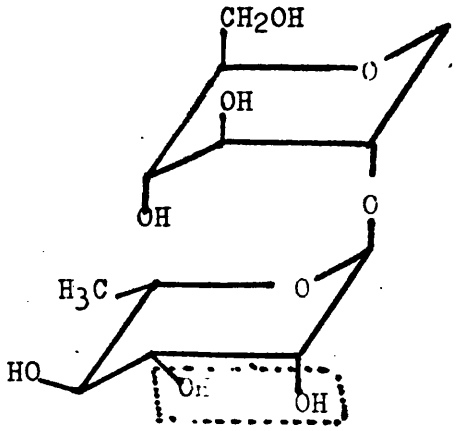
The interesting fact about dihydrochalcone sweetness is the fact that the molecule is so complex.A large dihydrochalcone aglycone is linked to a neohesperidose or glucose moiety and the nature of the substituents in the "B" ring is important.If receptor sites really are involved how many points of bonding with receptors are there? After all,there are six carbohydrate hydroxyl groups,three phenolic hydroxyl groups,a methoxyl group,a ketone group and two aromatic rings in neohesperidin DHC.If the carbohydrate groups interact with a receptor, why should the sweetness power be a thousand times sucrose? AH-B type bonding would be expected in only two pairs of hydroxyl groups in the neohesperidose moiety in which both the glucose and rhamnose are assumed to have the normal chair (Cl) conformation.This is predicted by Shallenberger's theory[135] that -OH groups in sugars are more likely to invoke sweetness if they have gauche (skew or staggered) conformations so that the inter-hydroxyl distance is ca. 3Å.



Neohesperidose
Cl conformation

If the --OH groups are eclipsed as is the case with vic-diol type pairs these bond intramolecularly,thus inhibiting bonding to a receptor and consequently reducing a sweetness effect.Similarly,if the -OH groups are in the anti conformation they are unable to act as an AH-B pair in concert.If we assume that the abnormal conformation is adopted,that is the inverse chair(IC) conformation,then only one pair of -OH groups would have the necessary gauche conformation.Incidentally, the primary -OH group of the glucose on C6 is also intramolecularly hydrogen-bonded to the pyranose ring oxygen atom,thus inhibiting its capability of exterior bonding.It is possible that the explanation for the non-sweetness of rutinose dihydrochalcones can be attributed to the bulky rhamnose molecule causing the glucose to adopt the IC conformation in order to relieve steric crowding around carbon atoms C5 and C6.The result would be that the -OH groups would adopt the anti conformation and would be ineffective as an AH-B pair.

It may be that the carbohydrate is not bonded to receptors at all but simply functions to keep the whole molecule oriented on the taste cell membrane.The carbohydrate part might face outward into the aqueous solution of the saliva so that the "B" ring and/or the dihydrochalcone aglycone can interact.It is worth speculating that a dihydrochalcone sophoroside(a 2-O-β-D-glucopyranosyl—D-glucopyranoside) would probably exhibit sweetness as would a dihydrochalcone rhamnoside.



Neohesperidose
1C conformation

Finally,it is worth speculating whether the slow build-up of the sensation of sweetness on the tongue wrought by dihydrochalcones is a consequence of the difficulty such a large molecule experiences in aligning itself onto its appropriate receptors on the taste cell membrane.In addition,the fact that dihydrochalcones impart a lingering sweetness may be the result of the relative difficulty for the bulky dihydrochalcone molecules to extricate themselves from their receptor sites.

Much work needs to be done in this area.The synthesis of dihydrochalcones containing methylated carbohydrate residues would narrow down the -OH groups which are responsible for sweetness,if any.

6) Patents.

U.S.Patent 3,087,821.[48] This is the first patent which Horowitz published on the synthesis of dihydrochalcones and their use as sweetening agents.

The compounds described were naringin DHC,neohesperidin DHC and prunin DHC. These were prepared from naturally-occurring flavanones by conversion to dihydrochalcones via chalcones.

U.S.Patent 3,375,242.[49] In this second patent of Horowitz two processes are described: 1) A process for preparing neohesperidin DHC by reacting naringin with an excess of isovanillin in hot aqueous alkali to produce neohesperidin chalcone and hydrogenating the separated chalcone to produce the dihydrochalcone. 2) A process for preparing neohesperidin DHC by reacting naringin with an excess of isovanillin in hot aqueous alkali to produce neohesperidin chalcone.The reaction mixture is then hydrogenated to produce the dihydrochalcone.

U.S.Patent 3,429,873.[50] Horowitz's third patent claims a process for preparing hesperetin DHC by treating hesperidin with alkali to produce hesperidin chalcone,hydrogenating the chalcone to hesperidin DHC and treating this dihydrochalcone with dilute acid to hydrolyse rhamnose from the rutinosyl radical so producing hesperetin DHC glucoside.

U.S.Patent 3,583,894.[136] The claims of this patent are similar to those described in the previous patent (U.S.3,429,873.) except that the acidic hydrolysis step is replaced by an enzymic hydrolysis step in the preparation of hesperetin DHC glucoside.The enzyme preparation is naringinase,the glucosidase component of which has been essentially inactivated.

U.S.Patent 3,364,196.[137] This patent claims a process for producing dihydrochalcones by hydrolysing flavanone glycosides to chalcones in alkaline solution and hydrogenating these chalcones to dihydrochalcones in the alkaline reaction medium.

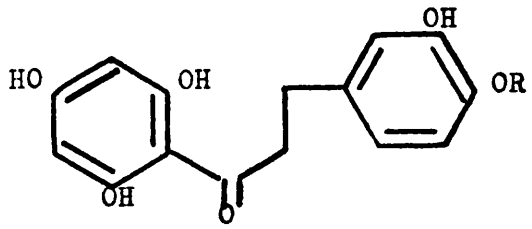
U.S.Patents 3,522,236. and 3,625,700.[138,139] These two patents are essentially identical except that the patent 3,522,236 makes only one claim, that is, the compound 2',4',6',3-tetrahydroxy-4-n-propoxydihydrochalcone-4'-β-neohesperidoside whereas the patent 3,625,700 also includes more specific uses of the compound in a sweetening composition.

G.B.Patent 1,189,573.[140] This patent claims a process of preparing chalcones by condensing phloroacetophenone-4-β-neohesperidoside and a 3-hydroxybenzaldehyde compound, namely isovanillin, 3-hydroxy-4-ethoxybenzaldehyde or 3-hydroxy-4-n-propoxybenzaldehyde solution. Dihydrochalcones are not covered by this patent.

G.B.Patent 1,216,047.[141] A sweetening composition is claimed in this patent for neohesperidin dihydrochalcone and saccharin in a ratio of from 0.1:1 to 3:1. Only food products are mentioned.

Fr.Patent 1558680.[142] This patent is essentially identical to the British patent 1,189,573.

Belgian Patent 773,258.[143] The sweetening composition contains 5 10^{-4}M to 2.0M concentration of a compound of formula:-



where R is Me, Et, Pr^i, Pr or Bu and an ingestible organic polar liquid solvent or a mixture of such liquids or with water; containing 0.15%w/w of the polar liquid. The composition may contain 1-99%w/w of a polyol (eg. sorbitol) in a 1:10 ratio with hesperetin DHC and 0.25-99%w/w of a sugar. The natural sweetness of sugar alcohols and glycerol is increased.

U.S.Patent 3653,923.[144] This patent is essentially identical to the G.B. patent 1,216,047.

7) Toxicology

The earliest report on dihydrochalcone toxicology was published[145] by the U.S. Department of Agriculture (USDA). Owing to the limited availability of the dihydrochalcones the dietary level used was only 0.5% in rat feeding trials and no adverse effects were noted.

Further trials were conducted by Mr.A.N.Booth of the Western Utilisation Research and Development Division, Agricultural Research Service, USDA.[146] In these tests naringin DHC and neohesperidin DHC were fed to weanling rats of both sexes at a 5% dietary level for as long as 170 days, which included the period of rapid growth and the added stress of reproduction. No abnormalities were detected as judged by growth, feed intake, haematology, reproductive performance, organ weights, gross pathology, photosensitivity, histopathology, urine analysis and water balance. It was concluded, therefore, that a concentration of 5% dihydrochalcone in the diet of rats is a "no effect" level, under these conditions. Unfortunately, there had been insufficient quantities of the dihydrochalcones to extend the feeding tests to the two-year duration required by the FDA.

In 1969, Clark reported[147] that naringin DHC had been fed in high levels to dogs to convince themselves that the animals would accept high levels in their diets. Then they decided to investigate neohesperidin DHC, which is sweeter and more soluble in water than naringin DHC, in a two-year pharmacological test using rats and dogs. In a personal communication[148] Mr.G.H.Robertson provided the information that the test on rats would be completed in September, 1972. The dietary levels used were 0.5, 2.5 and 5%. No serious adverse effects had been noted.

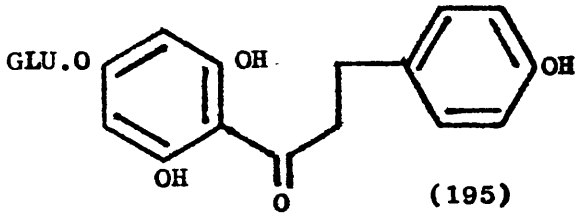
PART B

THE SYNTHESIS OF

DIHYDROCHALCONE GLUCOSIDES

DISCUSSION

The first sweet dihydrochalcones which were discovered were dihydrochalcone disaccharides, neohesperidosides in particular, although prunin dihydrochalcone is a sweet dihydrochalcone glucoside which also exhibits sweetness.



(195)

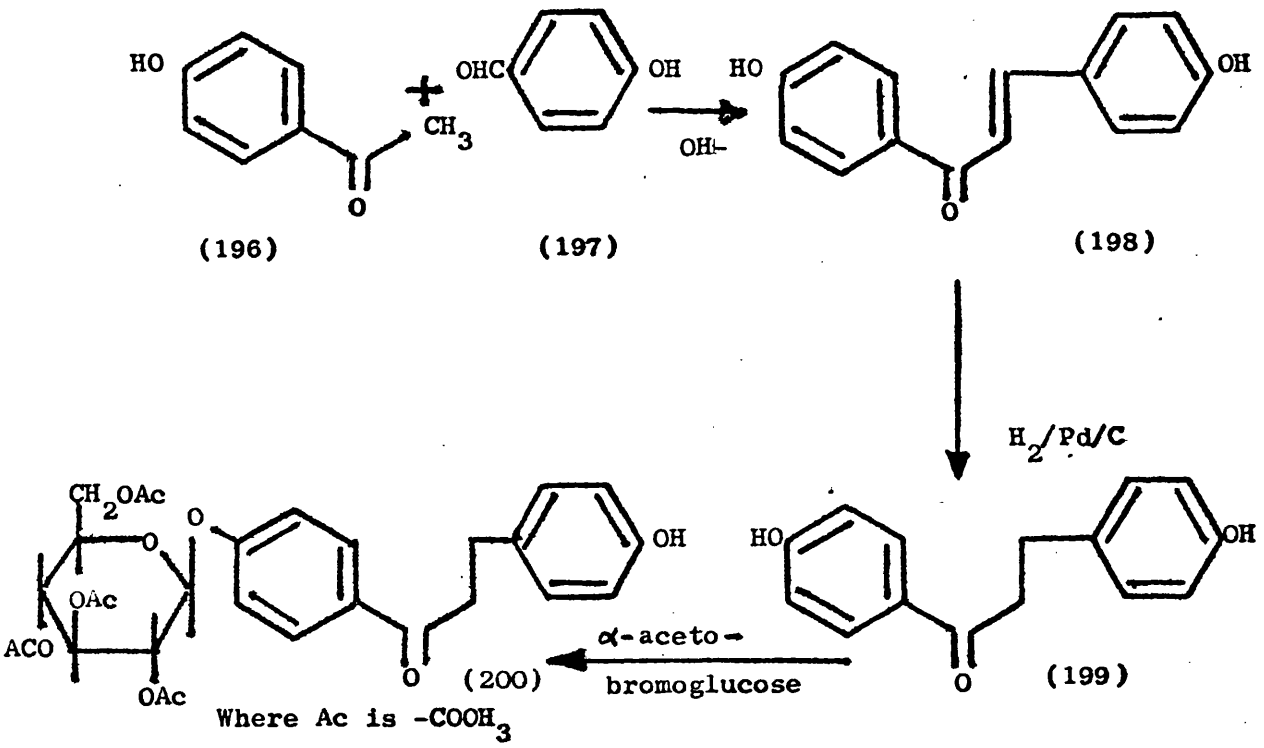
It was of interest to prepare simpler dihydrochalcones based not only on phloroacetophenone and resacetophenone structures for ring 'A', but based also on 4-hydroxyacetophenone. The syntheses of compounds which do not possess 2'- or 6'- hydroxy groups would yield compounds for sensory evaluation. If such compounds were sweet, then there may be advantages in basing possible commercial processes on the simpler compounds. Besides which it is of academic interest to know whether or not the phenolic -OH groups of the ring 'A' in dihydrochalcones contribute markedly to their sweetnesses.

As described in the Introduction (see Pages 22& 23), it is possible to prepare dihydrochalcone glucosides either by glycosylating the dihydrochalcone aglycone, or alternatively by glycosylating an appropriate ketone (tetraacetylpicein (122) for example) which is then reacted with a substituted benzaldehyde to form a chalcone. This chalcone in turn would be hydrogenated to the dihydrochalcone glucoside. The dihydrochalcone glycosylation method was tried first.

Dihydrochalcone Aglycone Glucosylation

4'-hydroxydihydrochalcones

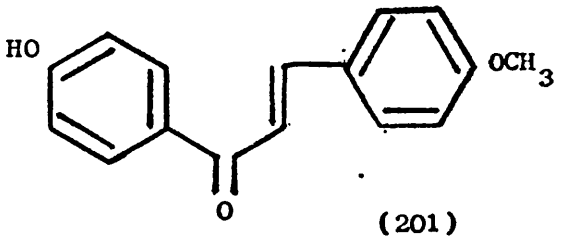
Thus 4-hydroxyacetophenone (196) was reacted with 4-hydroxybenzaldehyde (197) in alkaline solution to yield 4,4'-dihydroxychalcone (198). This chalcone was hydrogenated at room temperature and atmospheric pressure in the presence of 5% palladium/carbon catalyst to yield a brown oil. No crystals of 4,4'-dihydroxydihydrochalcone (199) were obtained.



α- acetobromoglucose (101) was prepared by acetylating D(+) glucose monohydrate to the pentaacetylderivative and by treating the resulting syrup containing pentaacetylglucose with hydrogen bromide gas. The method for the isolation of this compound is given in the experimental section (see page103). α- acetobromoglucose

was added to the brown oil which was obtained by hydrogenation of 4,4'-dihydroxychalcone (198). 4,4'-dihydroxydihydrochalcone-4'- (1-O-β-D-tetraacetylglucopyranoside) (200) was not obtained. No solid material could be isolated, only a brown, non-sweet oil remained.

Similarly, 4-methoxy-4'- hydroxychalcone (201) was obtained by treatment of 4-hydroxyacetophenone with anisaldehyde in alkaline solution. Hydrogenation of the chalcone yielded 4-methoxy-4'-hydroxydihydrochalcone (202). Treatment of the dihydrochalcone with α-acetobromoglucose yielded a non-sweet black oil. No crystalline material could be obtained.



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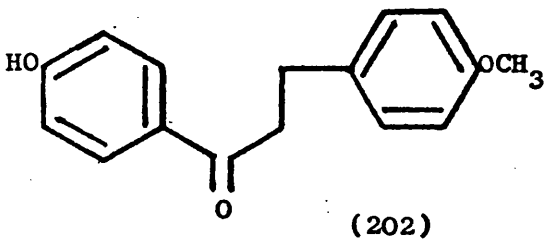

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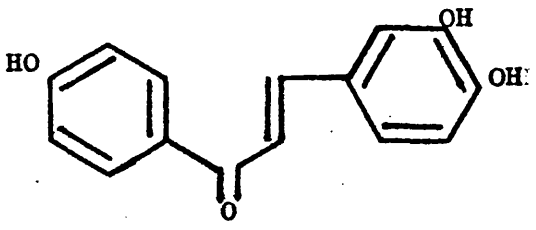
An attempt to prepare 3,4,4'-trihydroxychalcone (203) by reaction of 4-hydroxyacetophenone and protocatechuic aldehyde in alkaline solution yielded a black tar only.



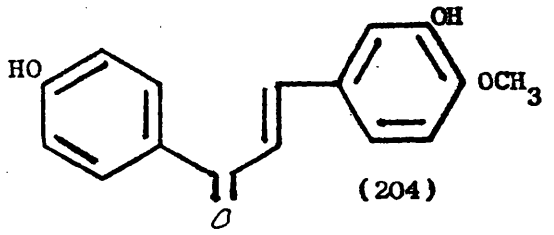
(203)

Several other chalcones and dihydrochalcones were prepared, but no glycosides could be prepared from the dihydrochalcones by treatment with α-acetobromoglucose. The compounds prepared were as follows:-

3,4'-dihydroxy-4-methoxychalcone (204) and 3,4'-dihydroxy-4-methoxydihydrochalcone (205)

4'-hydroxy-3,4-dimethoxychalcone (206) and 4'-hydroxy-3,4-dimethoxydihydrochalcone (207)

4'-hydroxy-3,4-methylenedioxychalcone (208) and 4'-hydroxy-3,4-methylenedioxydihydrochalcone (209)



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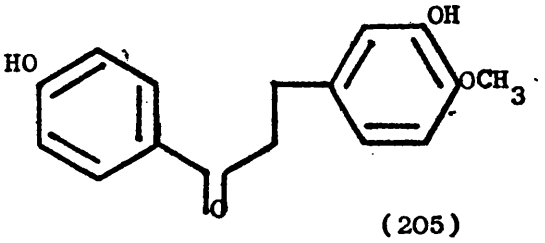

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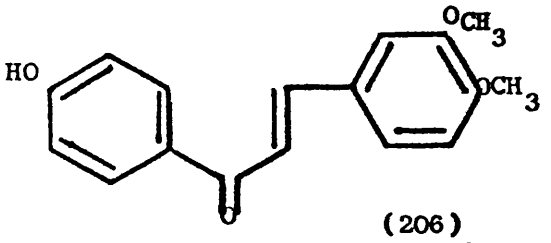

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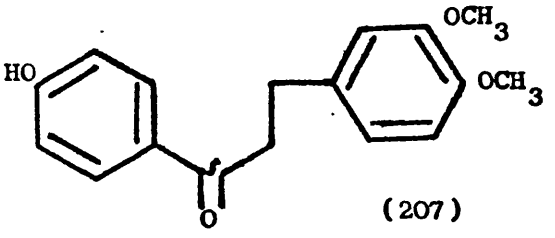

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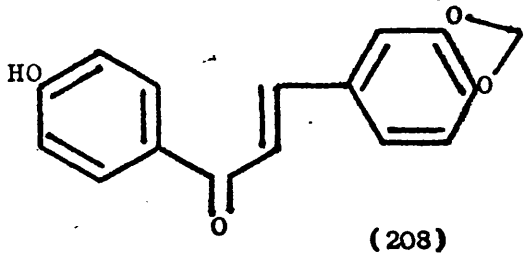

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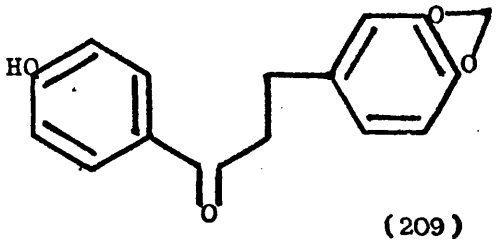
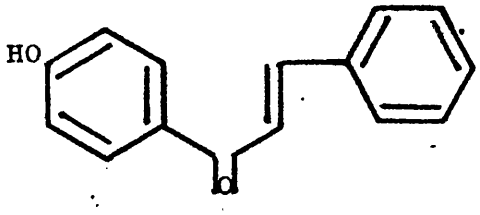


(209)

Only one of these syntheses which involved the reaction of a dihydrochalcone aglycone and α- acetobromoglucose was successful, i.e. the preparation of 4'-hydroxychalcone (210), the hydrogenation of the chalcone to the dihydrochalcone (211), and finally the preparation of 4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetylglucopyranoside) (212) in 22% yield by reaction of the dihydrochalcone (211) with α-acetobromoglucose.



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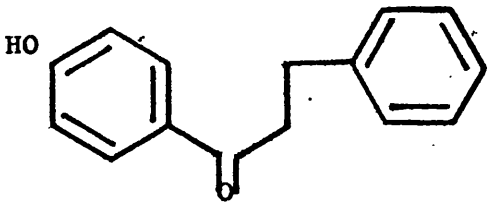

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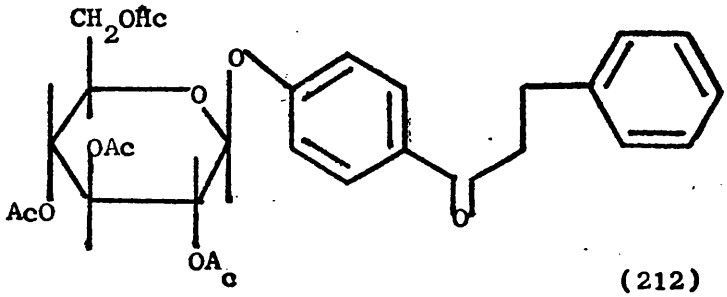
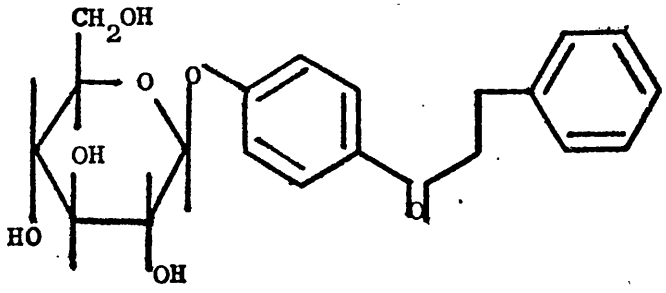


(212)

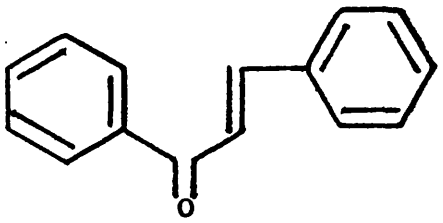
A 7.4% yield of compound (212) was also obtained by the reaction of 4'-hydroxydihydrochalcone with α-acetobromoglucose in the presence of mercuric acetate catalyst. The attempted hydrolysis of the tetraacetylglucoside (212) by treatment with sodium methoxide yielded not 4'-hydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (213) but rather the dihydrochalcone aglycone (211)



(213)

There is some doubt resting on this hydrolysis result. The product had a melting point of 154°C and the P.M.R. spectrum is entirely consistent with 4'-hydroxydihydrochalcone, but this latter compound has a m.p. of 60-61°C. It is likely that the required glucoside was formed, but that hydrolysis took place on warming the sample in the tube for the P.M.R. spectrum determination. This hydrolysis product of m.p. 154°C was non-sweet.

Three dihydrochalcone aglycones were also prepared from their respective chalcones in order to compare the rates of hydrogenation of these chalcones when using either palladium on charcoal catalyst or Adam's catalyst (PtO_2). Thus chalcone or benzylidene acetophenone (214) was hydrogenated to dihydrochalcone or 3-phenylpropiophenone (215).



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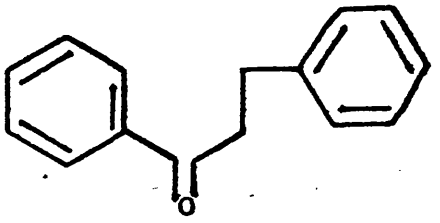
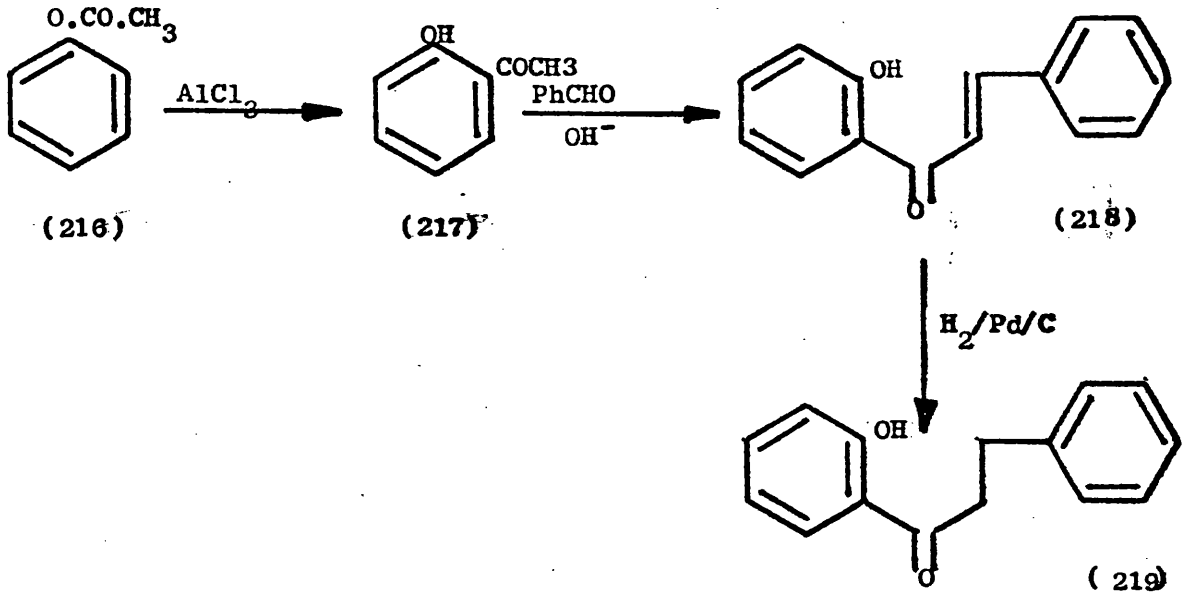


(215)

In order to prepare 2'-hydroxychalcone (218), 2-hydroxyacetophenone (217) was firstly prepared by acetylating phenol with acetyl chloride. Anhydrous aluminium chloride was then added in order to bring about a Fries re-arrangement of the phenyl acetate (216) to form 2-hydroxyacetophenone (217) which was converted to the chalcone (218) by a Claisen-Schmidt condensation with benzaldehyde.

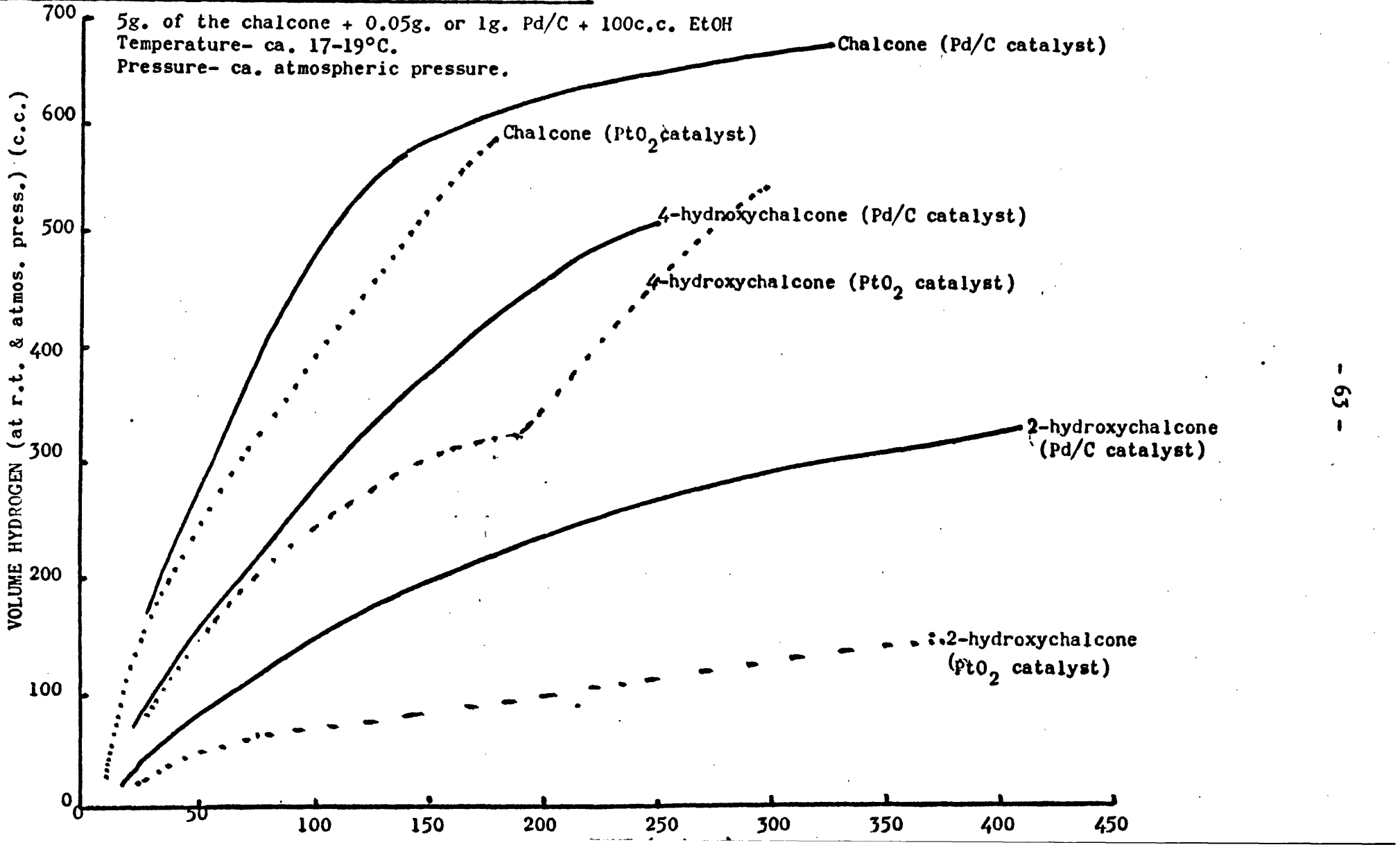


(216) (217) (218) (219)

Finally, the rate of hydrogenation of this chalcone to 2'-hydroxydihydrochalcone was measured by recording the volume of hydrogen which was taken up by the chalcone at various time intervals. (see Figure 2 , page 63).

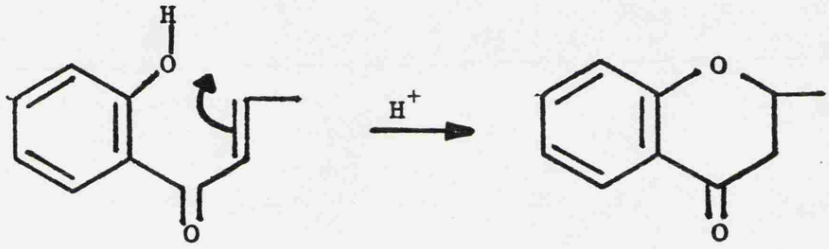
Similarly, 4'-hydroxychalcone (198) was prepared as described above and its rate of hydrogenation was also measured. The results of these hydrogenation are shown in Figure 2). The fastest hydrogenation was that of benzylidene acetophenone (214) when using palladium on charcoal catalyst. The second fastest hydrogenation was that of benzylidene acetophenone (214)when using Adam's catalyst. The descending order of the remaining combinations was 4'-hydroxychalcone (Pd/C catalyst) 4'-hydroxychalcone (PtO_2 catalyst), 2'-hydroxychalcone (Pd/C catalyst) and finally 2'-hydroxychalcone (PtO_2 catalyst), thus the order of ease of hydrogenation was :-

FIGURE 2 RATES OF HYDROGENATION OF SEVERAL CHALCONES.

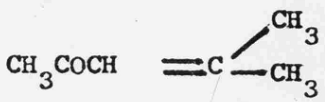


Chalcone > 4'-hydroxychalcone > 2'-hydroxychalcone.

Interestingly, the Adam's catalyst was more effective than the palladium/carbon catalyst, only in the case of the hydrogenation of 2'-hydroxychalcone. It is likely that the presence of the phenolic group on the 'A' ring is reducing the capacity of the double bond to donate electrons in forming new -C-H bonds. Thus, effect is even more marked in the case of 2'-hydroxychalcone which suggests that, in addition to electronic effects, the tendency for ring closure to occur diminishes the chalcones susceptibility to hydrogenation.



Because some initial difficulty had been experienced in achieving effective hydrogenations, some palladium/carbon catalyst was prepared at Bath by warming some washed, activated charcoal with palladous chloride and dilute hydrochloric acid. This slurry was added to a solution of sodium acetate in water and the whole mixture was hydrogenated for 16 hours. The catalyst was recovered by filtration, washed and dried. The activity of this catalyst was compared with that of commercially prepared material by measuring the rate of hydrogenation of mesityl oxide (220).



(220)

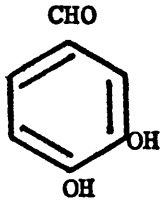
The commercial catalyst reached the theoretical hydrogen uptake after a hydrogenation time of 6 minutes, whereas, the catalyst prepared at Bath, took 15 minutes to reach the theoretical hydrogen uptake. The material prepared by the author was active, therefore, but it was not as active as the commercial material and better results for chalcone hydrogenations were obtained when the latter was used.

The reductions of several chalcones were attempted by using a zinc/acetic acid reagent, in the hope of replacing the time consuming hydrogenation method by a simpler procedure. Benzylidene acetophenone, 2'-hydroxychalcone and 4'-hydroxychalcone were each added to mixtures of water/ethanol/acetic acid and granulated zinc and each mixture was heated on a steam bath for a period of one hour. Benzylidene acetophenone yielded its dihydrochalcone (i.e. 3'-phenylpropiophenone (215)) in a 2.2% yield. 4'-hydroxychalcone (210) yielded a small amount of 4'-hydroxydihydrochalcone (211) whereas 2'-hydroxychalcone (218) yielded only a yellow, gum-like solid mass. This zinc/acetic acid method described above, is unsatisfactory as a reducing method for the chalcones described.

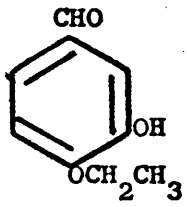
The use of the di-imide reaction as a method for the reduction of chalcones was also investigated. Benzylidene acetophenone, 2'-hydroxychalcone and 4'-hydroxychalcone were used for the trials. Each chalcone was added to 1% copper sulphate solution in water, and diluted with ethanol. Hydrazine hydrate followed by hydrogen peroxide were added and gums were obtained from each reaction mixture.

The di-imide reaction was also proved to be unsatisfactory for the reduction of the three chalcones described above.

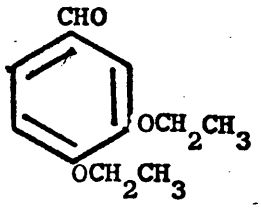
For the examination of various chalcones containing an ethoxy group in the 'B' ring 3'-hydroxy-4-ethoxybenzaldehyde (222) was prepared by the action of ethyl sulphate on protocatechuic aldehyde (221) in alkaline solution.



(221)



(222)



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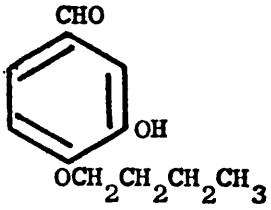
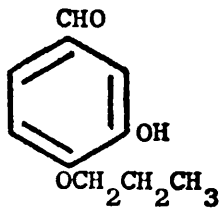


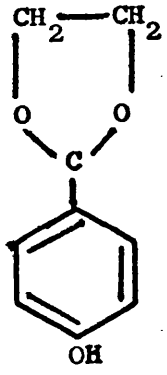
(224)



(225)

The monoethoxy derivative (222) was extracted into ether the diethoxy compound (223) was not isolated. The synthesis of 3 -hydroxy-4-(n-butoxy) benzaldehyde (223) was attempted by the refluxation of protocatechuic aldehyde in alkaline solution with n-butyl bromide. The residue obtained after evaporation of an ether extract yielded water only on vacuum distillation. Similarly, a synthesis of 3-hydroxy-4-(n-propoxy) benzaldehyde (225) was attempted by the refluxation of protocatechuic aldehyde in alkaline

solution with n-propyl bromide and resulted only with starting material. It was likely that the reason for the poor reactivity of the phenolic groups in the above reactions was caused by the reactivity of the aldehydic group causing tar formation. Therefore, it was decided to protect the aldehyde group by forming the acetal derivative. Therefore, 4-hydroxybenzaldehyde, together with ethylene glycol in benzene and containing a small quantity of toluene p-sulphonic acid, was heated under reflux on a Dean-Stark water separator for 12 hours. A large amount of a red, gummy jam-like material was obtained, the 4-hydroxybenzaldehyde, methylenedioxyacetal (226) was not obtained.



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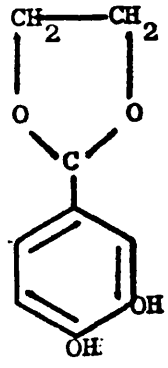


(227)

In the same way the synthesis of protocatechuic aldehyde, methylenedioxyacetal (227) was attempted by heating protocatechuic aldehyde with ethylene glycol in benzene as described for compound (226). The reaction product was a black, tarry mess and no attempt was made to isolate the acetal.

An alternative method for the synthesis of an alkoxy benzaldehyde is to prepare 4-(n-propoxy) benzoic acid (228) by the propylation of 4-hydroxybenzoic acid, to form the acid chloride (229) and finally to reduce the acid chloride to 4-(n-propoxy) benzaldehyde (230)

(228)

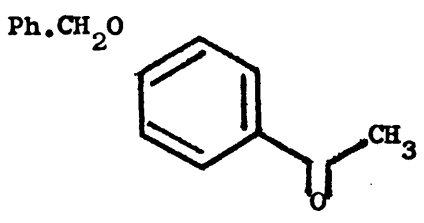
(229)

(230)

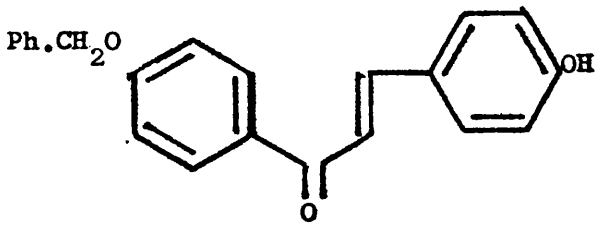
Thus 4-hydroxybenzoic acid was refluxed with n-propyl bromide to yield, after acidification, a white precipitate of 4-(n-propoxy) benzoic acid (228). Treatment of the 4-(n-propoxy) benzoic acid (228) with thionyl chloride failed to yield 4-(n-propoxy) benzoyl chloride (229).

The direct synthesis of 4-(n-propoxy) benzaldehyde (230) was attempted by heating 4-hydroxybenzaldehyde and n-propyl bromide under reflux. This procedure resulted in the formation of 4-(n-propoxy) benzoic acid (228), none of the aldehyde (230) was obtained.

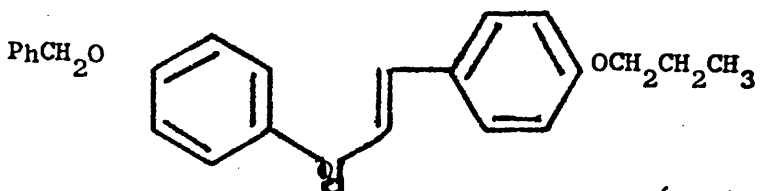
It was of interest to prepare 4-hydroxy-4'-benzyloxychalcone (232) in order that a propylation could be attempted on the 4-hydroxy group of the chalcone. Therefore, 4-hydroxyacetophenone and benzyl chloride in alkaline acetone were heated under reflux for three hours to yield 4-benzyloxyacetophenone (231).



(231)



(232)



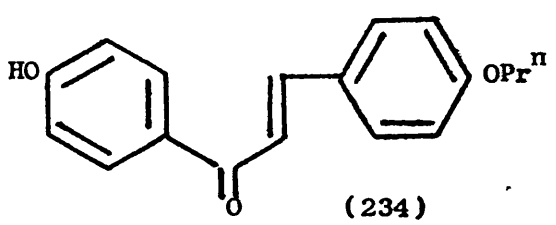
(233)

Compound (231) was dissolved in alkaline methanol/water, 4-hydroxybenzaldehyde added, and the mixture was heated under reflux for two hours. A solid was isolated which proved to be starting material i.e. 4-benzyloxyacetophenone (231) in 55% yield. This solid was redissolved in potassium hydroxide, and the solution extracted with diethylether to remove the 4-benzyloxyacetophenone. Acidification of the aqueous layer followed by extraction with diethylether yielded only 4-hydroxybenzaldehyde in a yield of ca. 6%.

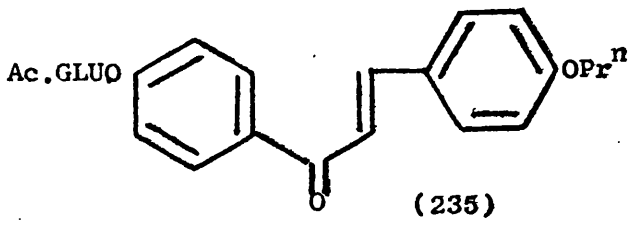
The synthesis was repeated except that following acidification, and ether extraction, of the reaction mixture, the ether layer was also washed with bisulphite solution in order to remove 4-hydroxybenzaldehyde starting material. After evaporation of the ether layer a white solid was obtained which was shown to be 4-benzyloxyacetophenone starting material recovered in 93% yield.

The synthesis was repeated a third time. The 4-benzyloxyacetophenone (231) and 4-hydroxybenzaldehyde were dissolved in alkaline methanol/water. This mixture was left at room temperature for three days and after extraction with ether, which was washed in the manner described above, an oil was obtained after evaporation of the ether. The chalcone 4-hydroxy-4'-benzyloxychalcone (232) was obtained in a yield of 11%.

The object of preparing this chalcone (232) was to prepare 4-(n-propoxy)-4'-benzyloxychalcone (233), the benzyl group protecting the 4'-hydroxy group from attack by the propyl moiety. The intention was to debenzylate this compound (233) by hydrogenation to form 4'-hydroxy-4-(n-propoxy) chalcone (234) and finally to form its



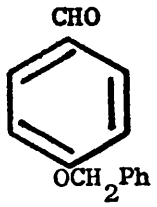
(234)



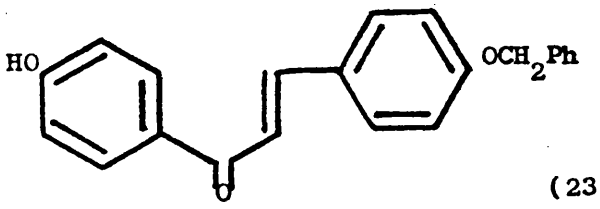
(235)

tetraacetylglucoside (235) by reaction with α-acetobromoglucose. However, the reaction of 4'-hydroxy-4-benzyloxychalcone with n-propyl bromide in alkaline solution failed to yield the expected propylated chalcone (233) and only starting material could be obtained. The fact that the propylation step failed to yield chalcone (233) meant that the syntheses of compounds (234) and (235) were excluded.

Another compound of interest is 4-benzyloxy-4'-hydroxychalcone (236) in order to prepare the 4'-glucoside of this chalcone without any competing reaction occurring at the hydroxy group in the 4-position Therefore, 4'-benzyloxybenzaldehyde (236) was prepared by the reaction of 4-hydroxybenzaldehyde and benzyl chloride.



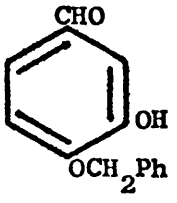
(236)



(237)

Then, 4'-benzyloxybenzaldehyde and 4-hydroxyacetophenone were reacted together in alkaline solution in an attempt to prepare 4-benzyloxy-4'-hydroxychalcone (237). A gum resulted from which was eventually obtained a yellow precipitate in 39% yield. The P.M.R. spectrum of this compound exhibited 5 benzylic protons, 4 aromatic protons (para substitution) and 2 methylene protons of the benzylic group. However, a single proton at δ = 2.6 remains unexplained. Perhaps the benzylic group has transferred to the 4-hydroxyacetophenone? In this case one would have expected 3 rather than 1 proton at a chemical shift of 2.6.

In order to prepare 3,4'-dihydroxy-4-benzyloxychalcone (239) a substituted benzaldehyde was firstly prepared i.e. 3-hydroxy-4-benzyloxybenzaldehy (238). This latter compound was made by reacting protocatechuic aldehyde and benzyl chloride in alkaline solution.



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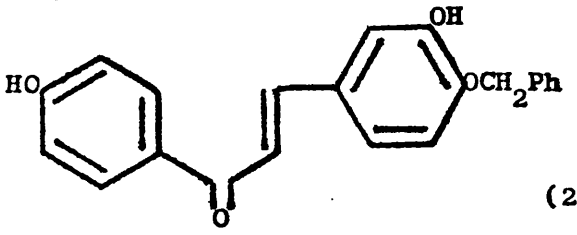
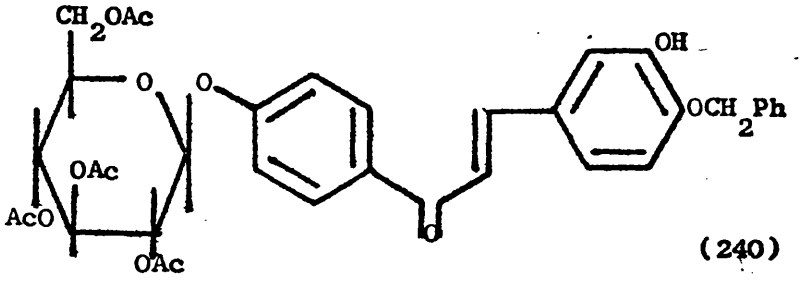


(239)

The chalcone (239) was prepared by reaction of 3-hydroxy-4-benzyloxybenzaldehyde and 4-hydroxyacetophenone in aqueous potassium hydroxide. The resulting gum was recrystallised from methanol to yield 11.5% of the chalcone. The synthesis of the glucoside of 3,4'-dihydroxy-4-benzyloxychalcone was attempted by reaction of the chalcone with α-acetobromoglucose in aqueous posassium hydroxide. 3,4'-dihydroxy-4-benzyloxychalcone-4'-(1-O-β-D-tetraacetylglucopyranoside) (239) was not isolated. A brown oil separated.



(240)

Treatment of this oil with various solvents, accompanied by cooling in the refrigerator failed to produce any solid material. Evaporation of the aqueous layer did not result in the precipitation of solid material.

Several dihydroxhalcone aglycones were examined for their possible formation of their respective glycosides. Each dihydrochalcone was added to quinoline together with freshly prepared silver carbonate. The mixture was stirred and α-acetobromoglucose was added and allowed to stand overnight. After filtration to remove silver oxide and rotary evaporation under reduced pressure of the filtrate, only dihydrochalcone starting material was obtained. The dihydrochalcones examined are listed below :-

4'-hydroxydihydrochalcone (241)

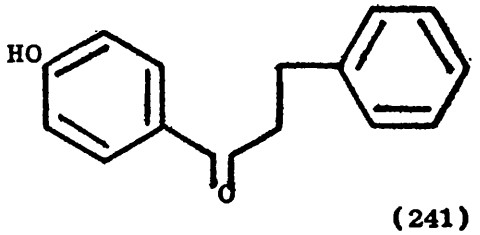
4'-hydroxy-3,4 -dimethoxydihydrochalcone (242)

4'-hydroxy-4-methoxydihydrochalcone (243)

4'-hydroxy-3,4 -methylenedioxydihydrochalcone (244)

3,4'-dihydroxy-4-methoxydihydrochalcone (245).

The structures of these dihydrochalcones are shown below :-



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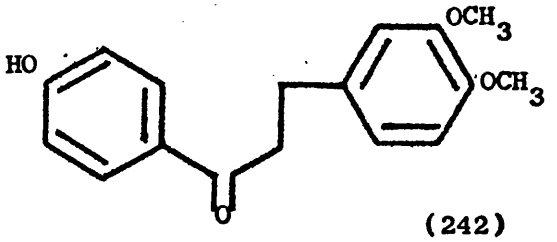

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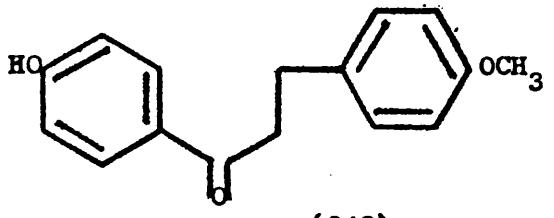
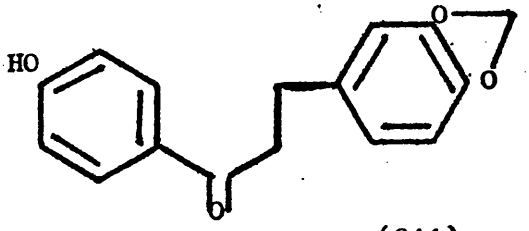


(243)



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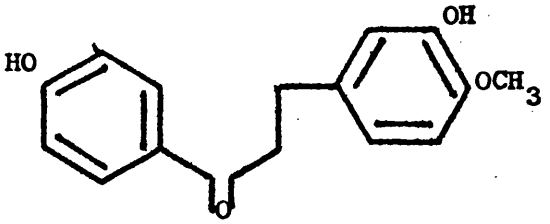
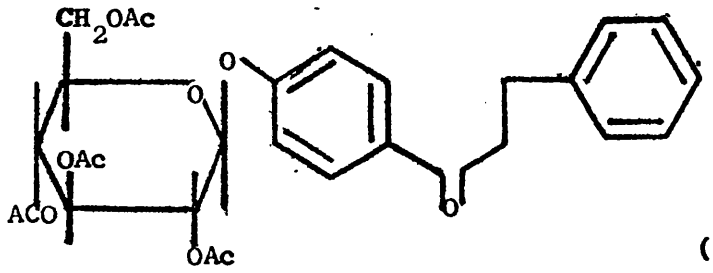


(245)

The reactions with α-acetobromoglucose were intended to form the respective dihydrochalcone glucoside. Thus, 4'-hydroxydihydrochalcone might have yielded 4'-hydroxydihydrochalcone-4-(1-O-β-D-tetraacetylglucopyranoside (246), but none was in fact obtained.



(246)

Dihydrochalcone Aglycone Glucosylation

2) 2',4'-dihydroxydihydrochalcones

2',4'-dihydroxyacetophenone (resacetophenone) was reacted with the appropriate substituted benzaldehyde. All the reactions which were tried gave brown gummy residues excepting the case of the reaction of 3,4-dimethoxybenzaldehyde with resacetophenone when a yellow precipitate of 2',4'-dihydroxy-3,4-dimethoxychalcone (247). The substituted benzaldehydes which were reacted with resacetophenone but which did not yield chalcones were as follows :-

benzaldehyde,4-hydroxy-benzaldehyde,4-methoxybenzaldehyde (anisaldehyde) 3-hydroxy-4-methoxybenzaldehyde (isovanillin) and 3,4-methylenedioxybenzaldehyde (piperonal). The chalcones which were not isolated were :-

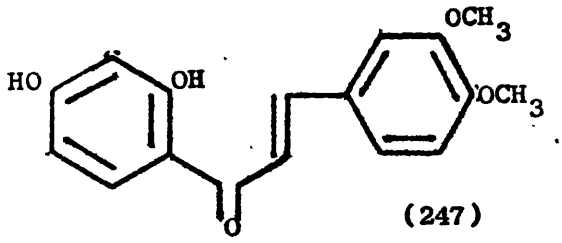
2; 4'-dihydroxychalcone (248)

2',4 ,4'-trihydroxychalcone (249)

2',4'-dihydroxy-4-methoxychalcone (250)

2',3 ,4'-trihydroxy-4-methoxychalcone (251)

2',4'-dihydroxy-3,4-methylenedioxychalcone (252)



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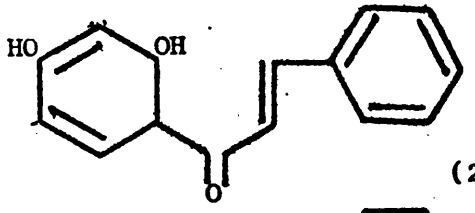

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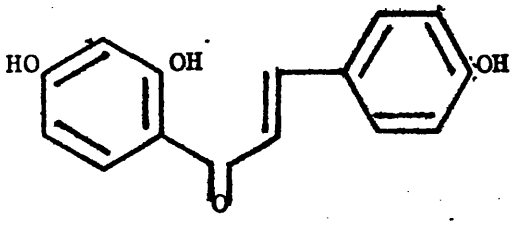

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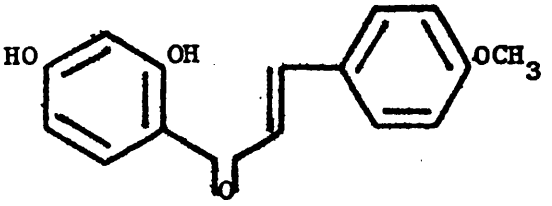
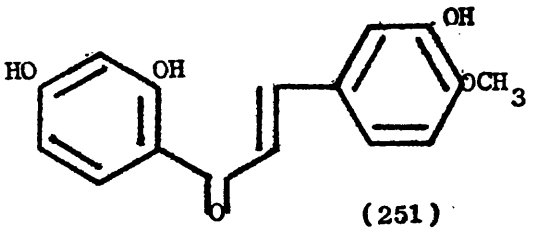
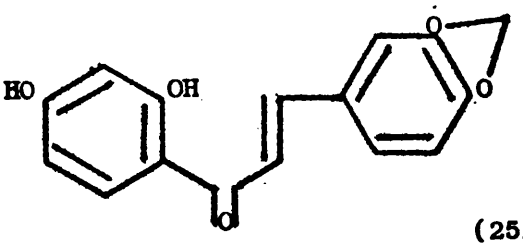


(250)



(251)



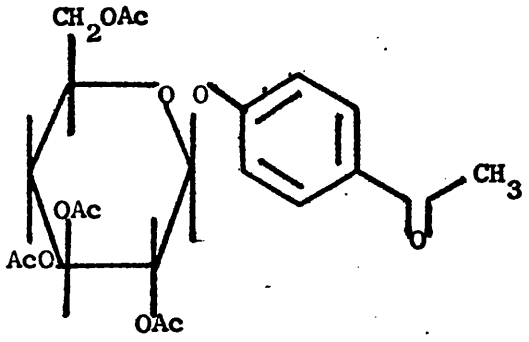
(252)

The hydrogenation of 2',4'-dihydroxy-3,4-dimethoxychalcone (247) was carried out with the object of preparing 2',4'-dihydroxy-3,4-dimethoxydihydrochalcone (252a) A brown oil was finally obtained from which no solid material could be isolated.

The Preparation of Dihydrochalcone Glucosides from Tetraacetylpicein

The methods described above for the attempted syntheses of dihydrochalcone glucosides from the dihydrochalcone aglycones were not successful except in the case of 4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetyl-glucopyranoside) (212) The alternative route of firstly preparing the glucoside of 4-hydroxyacetophenone was adopted, therefore.

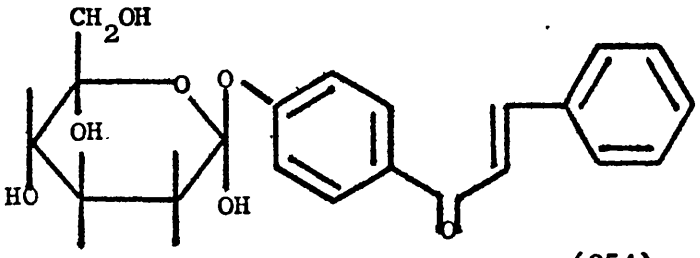
Tetraacetylpicein or 4-hydroxyacetophenone-4-(2,3,4,6-tetraacetyl-1-O-β-D-glucopyranose) (253) was prepared in 18 - 38% yield by the reaction of 4-hydroxyacetophenone and α-acetobromoglucose in alkaline solution.



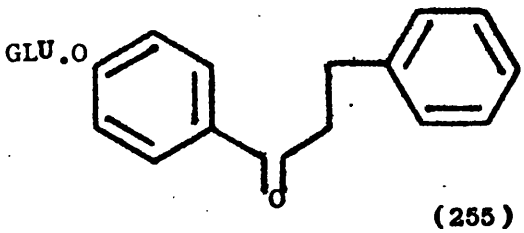
(253)

In an attempt to prepare tetraacetylpicein by reacting 4-hydroxyacetophenone and α-acetobromoglucose in methylene dichloride and using silver carbonate as the catalyst, unchanged α-acetobromoglucose was recovered and tetraacetylpicein was not isolated.

Several chalcones were prepared using the tetraacetylpicein as starting material, thus, reaction with benzaldehyde yielded 4'-hydroxychalcone-4'-(1-O-β-D-glucopyranoside) (254). Hydrogenation of the chalcone yielded a pale yellow gum from which 4'-hydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (255) could not be isolated.



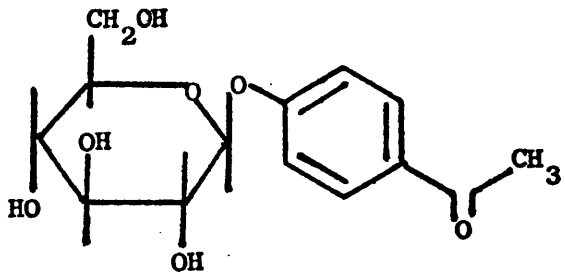
(254)



(255)

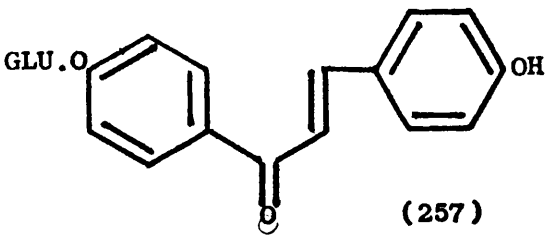
When tetraacetylpicein (253) was reacted with 4-hydroxybenzaldehyde there was no instant development of the yellow colour which is characteristic of chalcone formation but colourless, crystalline needles of picein (256)

separated, its structure confirmed by comparison of its m.p. with that quoted by Mauthner.[65]

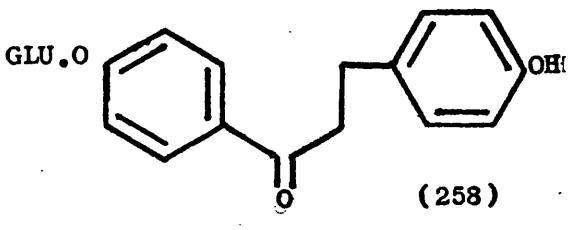


(256)

The preparation was repeated and the solution became dark yellow in colour after standing at room temperature for six days. A yellow gummy solid was finally obtained whose N.M.R. spectrum is consistent with that expected for 4,4'-dihydroxychalcone-4'-(1-O-β-D-glucopyranoside) (257). Hydrogenation of this yellow gum at room temperature and at atmospheric pressure using a 5% palladium on carbon catalyst failed to yield crystalline 4,4'-dihydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (258). The gummy residue was not sweet.



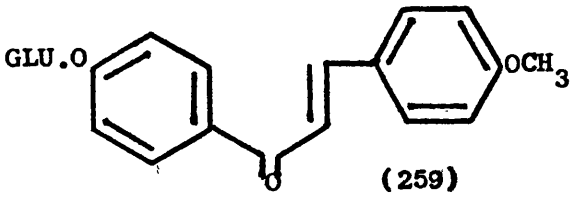
(257)



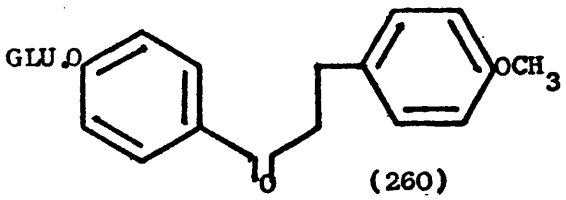
(258)

In a similar fashion, the reaction of tetra-acetylpicein and 4-methoxybenzaldehyde (anisaldehyde) gave 4'-hydroxy-4 -methoxychalcone-4'-(1-O-β-D-glucopyranoside) (259), a pale yellow solid. Hydrogenation

of chalcone (259) gave 4'-hydroxy-4-methoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (260) (see spectrum), a pale yellow non-sweet, wax-like solid.

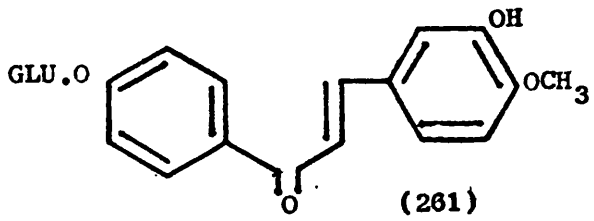


(259)

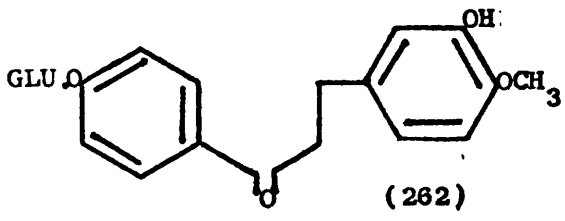


(260)

The result of the reaction of tetraacetylpicein and 3-hydroxy-4-methoxybenzaldehyde (isovanillin) in alkaline solution was 3,4'-dihydroxy-4-methoxychalcone-4'-(1-O-β-D-glucopyranoside) (261). Hydrogenation of this chalcone yielded a pale brown,non-sweet gum of 3,4'-dihydroxy-4-methoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (262).

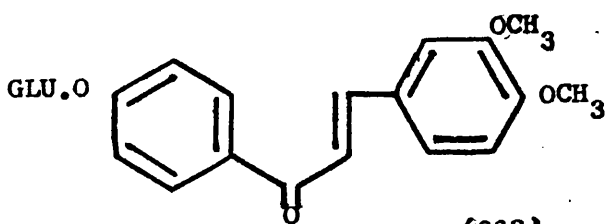


(261)

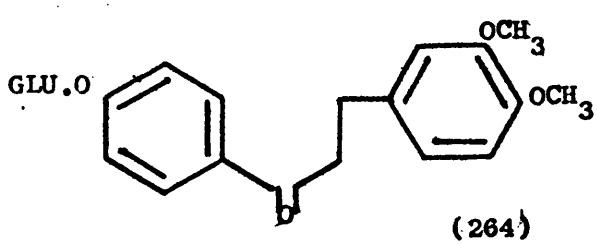


(262)

When tetraacetylpicein and 3,4-dimethoxybenzaldehyde were left to stand for six days aqueous alkali, on neutralisation the chalcone, 4'-hydroxy-3,4 -dimethoxy-4'-(1-O-β-D-glucopyranoside) (263), was obtained as a bright yellow gum. Hydrogenation of the chalcone gave a pale brown, non-sweet gum. The dihydrochalcone, 4'-hydroxy-3,4 -dimethoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (264) was not obtained as a crystalline solid.

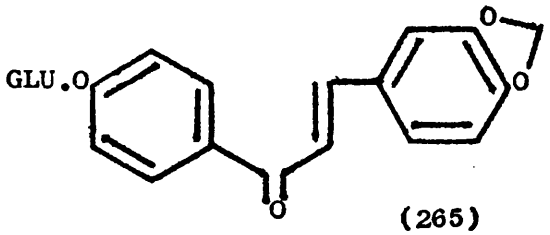


(263)

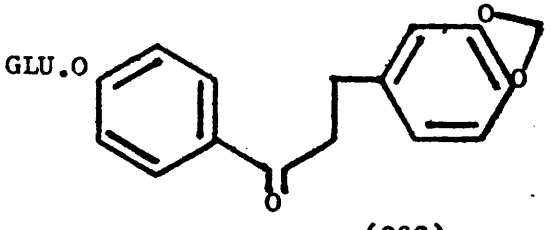


(264)

4'-hydroxy-3,4 -methylenedioxychalcone-4'-(1-O-β-D-glucopyranoside) (265) was obtained as a yellow crystalline chalcone after reaction of tetraacetylpicein and piperonal. Following hydrogenation a white, non-sweet solid was obtained of 4'-hydroxy-3,4 -methylenedioxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (266) (see spectrum 4).

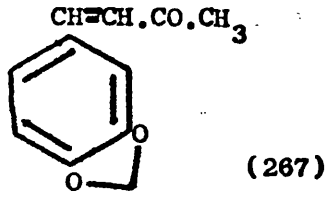


(265)

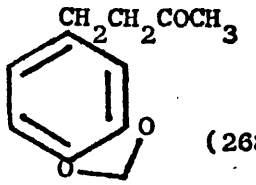


(266)

In a second attempt at the preparation of chalcone (265) the reaction was carried out in acetone and compound (267) was formed which on hydrogenation yielded compound (268), which serves to demonstrate that one must beware of the interactions of a solvent.



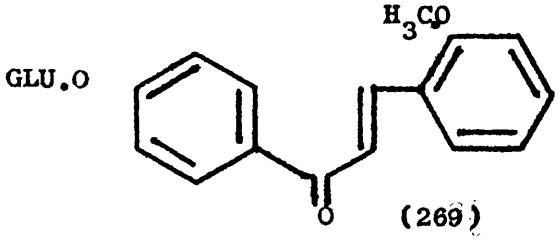
(267)



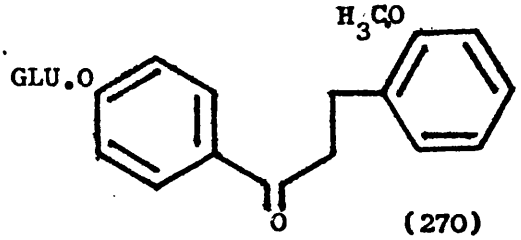
(268)

Incidentally, the only suitable recrystallisation solvent system found for these chalcone glucosides were either ethanol or methanol, being slightly soluble in the cold and soluble in the hot solvent.

Also prepared from tetraacetylpicein was 2-methoxy-4'-hydroxychalcone-4'-(1-O-β-D-glucopyranoside) (269) and its dihydrochalcone as a pale yellow non-sweet syrup (270)



(269)

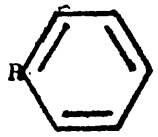
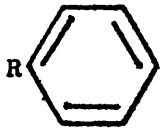
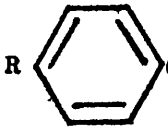
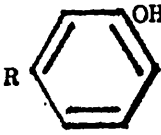
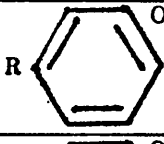
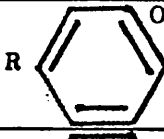
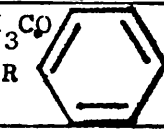


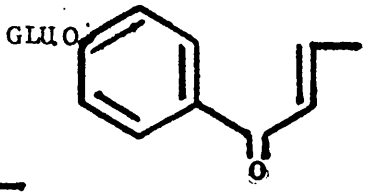
(270)

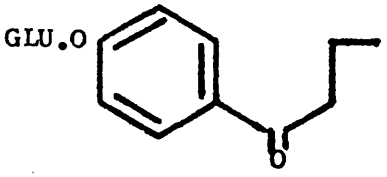
Finally, the chalcones, glucosides and dihydrochalcone glucosides which were obtained are summarised in Table 7 of the dihydrochalcone glucosides compounds 255, 258, 262 and 264 were obtained as gums only compound 260 and compound 266 were obtained as solids. All these reaction products were found to be non-sweet.

TABLE 7

CHALCONE & DIHYDROCHALCONE GLUCOSIDES PREPARED BY THE AUTHOR AND THEIR SWEETNESSES

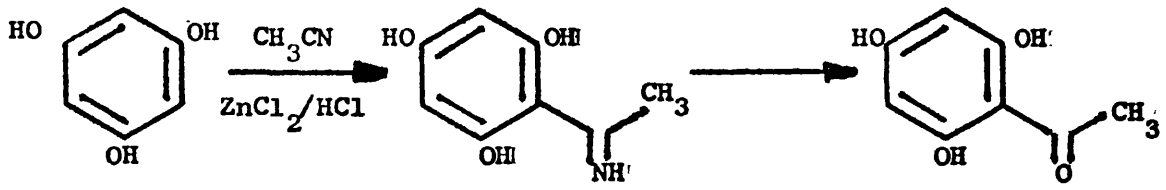
Compound No	Structure	Appearance of chalcone glucosides	Appearance of dihydrochalcone glucosides	Sweetness
254 and 255		Pale yellow gum	Pale yellow gum	Non-sweet
257 and 258		Yellow gummy solid	Gummy residue	-ditto-
259 and 260		Pale yellow solid	Pale yellow waxy solid	-ditto-
261 and 262		Gummy solid	Pale brown gum	-ditto-
263 and 264		Bright yellow gummy solid	Pale brown gum	-ditto-
265 and 266		Yellow crystals	White crystals	-ditto-
269 and 270		Bright yellow solid	Brown gum	-ditto-

In the above Table R is :-  for the chalcones

and  for the dihydrochalcones.

The Glucosylation of Phloroacetophenone

Phloroacetophenone (273) was prepared by reaction of phloroglucinol (271) with acetonitrile in diethylether solution with zinc chloride as catalyst. Hydrogen chloride gas was bubbled through the mixture. The resulting ketimine hydrochloride (272) was hydrolysed by boiling water to form phloroacetophenone (273).



(271) (272) (273)

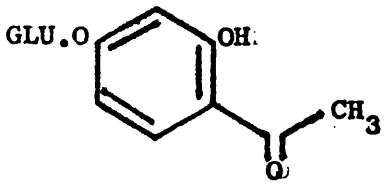
The synthesis of phloroacetophenone-4'-(1-O-β-D-glucopyranoside) (137) was attempted by reaction of phloroacetophenone with α-acetobromoglucose in alkaline solution. A precipitate was collected but its melting point of 84°C was not the value quoted by Ollis[149] & Zemplen[150] of 215-19°C. The N.M.R. spectrum seems consistent with that expected for phloroacetophenone glucoside but it is likely that this spectrum was the result of making a spectrum determination on a mixture of phloroacetophenone and 2,3,4,6-tetraacetylglucopyranose.

A comparison was made of Zemplen's methods using either potassium hydroxide or silver carbonate in the attempted synthesis of phloroacetophenone glucoside. In order to assess the former method, phloroacetophenone and α-acetobromoglucose were dissolved in acetone and potassium hydroxide, dissolved in water, was added.

A cream-coloured, gummy solid resulted from which no crystals could be obtained. Thin layer chromatography showed a single spot corresponding to starting material.

When Zemplen's second method was used in which phloroacetophenone and α-acetobromoglucose were stirred overnight with silver carbonate, no crystals of phloroacetophenone glucoside separated. This layer chromatography showed two spots, however, one spot corresponding to that of phloroacetophenone and the second probably being due to the glucoside.

A similar comparison was made of Zemplen's methods using either potassium hydroxide or, alternatively, silver carbonate catalyst for the reaction of resacetophenone with α-acetobromoglucose. As in the case of phloroacetophenone no crystals of resacetophenone-4'-(1-O-β-D-glucopyranoside) (272) were obtained. Thin layer chromatography showed only a tiny spot at an Rf value of 0.75 which is likely to be due to the glucoside.



(274)

The silver carbonate method resulted in a reaction product which produced a far larger spot at this Rf value.

The Synergistic Increase in Sweetnesses produced by Dihydrochalcone Aglycones with Several Sweeteners

The dihydrochalcones examined were :-

1. 4'-hydroxydihydrochalcone (211)
2. 4'-hydroxy-4-methoxydihydrochalcone (202)
3. 4'-hydroxy-3,4 -methylenedioxydrochalcone (209)
4. 3,4'-dihydroxy-4-methoxydihydrochalcone (205)
5. 4'-hydroxy-3,4 -dimethoxydihydrochalcone (207)

The dihydrochalcone was added to a 5% w/v solution of glucose, sucrose, sorbitol or mannitol so that the solution was 0.05M with respect to the dihydrochalcone. The sweetnesses of the sweetener solution alone and together with the various dihydrochalcones were assessed by the author. These observations, together with comments on side effects appear in Table 8 on page 83.

3,4'-dihydroxy-4-methoxydihydrochalcone produced an enhanced sweetness sensation when added to 5% w/v solutions of either glucose, sorbitol or mannitol. It produced a simultaneous burning sensation on the tongue. The other four dihydrochalcones produced no sweetness enhancement. It is worth noting that this dihydrochalcone has the same structure in the 'B' ring as hesperetin dihydrochalcone and neohesperidin dihydrochalcone.

TABLE 8

THE EFFECT OF THE ADDITION OF SEVERAL DIHYDROCHALCONES TO SWEETNENER SOLUTIONS

	5% Sucrose in Water	5% Glucose in Water	5% Sorbitol in Water	5% Mannitol in Water
4'-hydroxy DHC (211)	No Sweetness enhancement	No sweetness enhancement	No sweetness enhancement	No sweetness enhancement
4'-hydroxy-4-methoxy DHC (202)	Very slight sweetness enhancement	Very slight sweetness enhancement	-ditto-	-ditto-
4'-hydroxy-3,4-methylenedioxyDHC (209)	Very slight sweetness enhancement. Sl. burning sensation on tongue.	No sweetness enhancement	No sweetness enhancement. Burning sensation on the tongue.	No sweetness enhancement
3,4'-hydroxy-4-methoxy DHC (205)	No sweetness enhancement. Marked burning sensation on the tongue	Strong sweet sensation.	Enhancement of sweetness. Simultaneous bitterness and sl. burning sensation.	Enhancement of sweetness
4'-hydroxy-3,4-dimethoxy DHC (207)	No sweetness enhancement	No sweetness enhancement	No sweetness enhancement. Slight burning sensation.	No sweetness enhancement.

EXPERIMENTAL

The Preparation of Dihydrochalcone Glucosides from their Dihydrochalcone Aglycones.

4,4'-dihydroxychalcone (198).

4-hydroxyacetophenone(27.2g,200mM) and 4-hydroxybenzaldehyde (24.2g,200mM) were added to 160cc. of 20%w/v potassium hydroxide in water.The mixture was heated for 3 hours on a steam bath then added to 600cc. cold water. 80cc. concentrated hydrochloric acid were poured into this when a dark yellow oil resulted as the bottom layer.After storing in a refrigerator overnight the oil remained-nc crystallisation had taken place.The aqueous layer was decanted off and 200cc. methanol added after which a precipitate was thrown down.An oily solid was collected which after dissolution in methanol and storage in a refrigerator did not yield a solid,even after 2 days storage.A second attempt resulted in a 9% yield of the chalcone. m.p. 191-5°C (lit.[151,152] 197°C, 200°C. λ_{max}(ϵ)nm., in neutral methanol, 348 (26,200), in 0.01M KOH/MeOH, 474 (39,800). ν_{max} $cm.^{-1}$, 3300, 1645, 1590, 1375, 1345, 1220, 1165, 1105, 1030, 970, 805.

The AttemptedSynthesis of4,4'-dihydroxydihydrochalcone(199).

4,4'-dihydroxychalcone (3g,12.5mM) was dissolved in 50cc. methanol and 1g. of 10% palladium on carbon catalyst was added.The mixture was hydrogenated at room-temperature and at atmospheric pressure overnight.After fitration and rotary-evaporation, followed by overnight storage in the refrigerator, the brown oil failed to crystallise.

The Attempted Synthesis of 4,4'-dihydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside (200).

The oil resulting from the 4,4'-dihydroxydihydrochalcone preparation (3g,ca.12.4mM) was added to 100cc. 10% water in acetone,together with 1.4g. potassium hydroxide. α-acetobromoglucose (5.1g,15.4mM) was added slowly during 5 minutes. Initially a brown oil was obtained which turned pale yellow as the α-acetobromoglucose was added.Rotary-evaporation and cooling yielded a brown,non-sweet oil which failed to crystallise.

4-methoxy-4'-hydroxychalcone(201).

4-hydroxyacetophenone (13.6g,100mM)and anisaldehyde (13.6g,100mM) were added to 80cc. of 20% potassium hydroxide in water and the mixture was heated for 3 hours on a steam-bath.The mixture was poured into 300cc. of water and 40cc. of concentrated hydrochloric acid added. A yellow gum was precipitated which turned crystalline in less than 5 minutes.This solid was recrystallised from its solution in 500cc. methanol to yield 15.2g.of the chalcone(60%). m.p. 184-188°C (lit.[153] 187°C). λ_{max} 343nm., ε=30,100 in methanol. λ_{max} 388nm., ε=29,600 in 0.1M KOH/MeOH. ν =3140cm.$^{-1}$ (-OH) and 1645cm.$^{-1}$ (>C=O). 1600, 1560, 1510, 1280, 1250, 1215, 1170, 1160, 1030, 1015, 985, 830, 810.

4-methoxy-4'-hydroxydihydrochalcone(202).

4-methoxy-4'-hydroxychalcone (15.2g,60mM) was dissolved in 500cc. methanol and 1g. 10% palladium on carbon catalyst was added.The mixture was hydrogenated at room-temperature and atmospheric pressure.Theoretical uptake 1340cc.,actual uptake 1465cc. Yield of solid 12.7g (83%) after filtration, rotary-evaporation,cooling and drying.m.p. 72-80°C. λ_{max} in methanol 277nm. (shoulder at 283nm.) ε =3260 . λ_{max} in 0.1M KOH/MeOH 284nm., ε =7700(shoulder at 278nm.). ν =3420,3300 and 3200 cm.$^{-1}$ (-OH) and 1620 cm.$^{-1}$ (>C=O). 1600, 1510, 1300, 1270, 1240, 1075, 1030, 935, 920, 825.

The Attempted Synthesis of 4-methoxy-4'-hydroxydihydrochalcone-4'-β-D-glucopyranoside (275).

4-methoxy-4'-hydroxydihydrochalcone (2g,7.8mM) was added to a solution of 0.8g. potassium hydroxide in 50cc. of 10% water in acetone. α-acetobromoglucose (3.2g,9.6mM) was added slowly during 5 minutes when the colour of the solution changed from pale yellow to dark brown and a lower layer of a black oil formed.The oil remaining after rotary-evaporation had no sweet taste and no solid was obtained after attempted crystallisations with various solvents or by drying in a vacuum-dessicator.

3,4,4'-trihydroxychalcone (203).

4-hydroxyacetophenone (13.6g,100mM) and protocatechuic aldehyde (13.8g, 100mM) were added to 80cc. 20% potassium hydroxide in water and heated on a steam-bath for 3 hours.Black tarry product was obtained and no chalcone could be isolated.

3,4'-dihydroxy-4-methoxychalcone (204).

4-hydroxyacetophenone (13.6g,100mM) and isovanillin (15.2g,100mM) were added to 80cc. 20% potassium hydroxide in water andheated on a steam-bath for 3 hours.The reaction mixture was poured into 300cc. water and acidified with 40cc. concentrated hydrochloric acid to yield a yellow gum which turned crystalline in less than one minute.This precipitate was re-crystallised from 400cc. methanol.Yield of chalcone (1st.crop)-7.61g.(28.2%), (2nd.crop)-2.91g.(10.8%).A repeat synthesis yielded an 83% yield. m.p.208°C. λ_{max} in neutral methanol-358nm., ε =25,100, λ_{max} in 0.1M KOH/MeOH -402nm., ε =38,400. ν =3480,3180 $cm.^{-1}$ (-OH) and 1650 $cm.^{-1}$ ($\rangle C=O$).1610, 1595, 1580, 1550, 1510, 1265, 1155, 1120, 1030, 1020, 970, 825, 790, 690.

3,4'-dihydroxy-4-methoxydihydrochalcone (205).

3,4'-dihydroxy-4-methoxychalcone (10.5g,38.6mM) was dissolved in 100cc. methanol and 1g. 10% palladium/charcoal catalyst was added.The mixture was hydrogenated overnight at room-temperature and atmospheric pressure,then filtered to remove the catalyst.Rotary evaporation yielded a grey oil which solidified after leaving for two days in the refrigerator .The solid was re-crystallised from methanol to yield a white solid. m.p.62-64°C. λ_{max} in neutral methanol 279nm., ε =5020, λ_{max} in 0.1M KOH/MeOH 295nm., ε =10,000. ν =3300$cm.^{-1}$ (-OH),1665$cm.^{-1}$ ($\rangle C=O$). 1600, 1575, 1510, 1435, 1355, 1275, 1215, 1160, 1125, 1020, 955, 830.

The Attempted Synthesis of 3,4'-dihydroxy-4-methoxydihydrochalcone-4'-β-D-glucopyranoside (276).

3,4'-dihydroxy-3-methoxydihydrochalcone (4g,14.7mM) was dissolved in a solution containing 1.7g potassium hydroxide in 10% water in acetone.

α-acetobromoglucose (6.1g,18.4mM) was added slowly over 5 minutes at room temperature.A brown oily layer formed at the bottom of the flask.After rotary-evaporation and cooling a brown sludge was obtained from which brown crystals (0.9g.) were isolated on recrystallisation from methanol.These crysals showed a peak at 1750cm.$^{-1}$ (OAc) but a carbonyl peak at ca. 1650cm.$^{-1}$ was absent. The brown oil had little odour and it possessed a peculiar taste character which was neither sweet nor bitter but rather anaesthetic.

4'-hydroxy-3,4-dimethoxychalcone (206).

4-hydroxyacetophenone (13.6g,100mM) and 3,4-dimethoxybenzaldehyde (16.6g, 100mM) were dissolved in 80cc. of 20% potassium hydroxide in water and the mixture was heated for 3 hours on a steam-bath then added to 300cc. cold water and 40cc. concentrated hydrochloric acid stirred in. A yellow gum formed which crystallised after 15 minutes standing at room-temperature. This solid was recrystallised from 800cc. methanol.Yield 16.85g (59.4%).m.p.196-200°C, (lit[154] 208°C). λ_{max} in neutral methanol 354nm., ε=24,600, λ_{max} in 0.1M KOH/MeOH 392nm., ε=34,700. ν=3180cm.$^{-1}$ (-OH) and 1635cm.$^{-1}$ ($\rangle$C=O). 1600, 1550, 1505, 1285, 1260, 1210, 1155, 1040, 1025, 985, 830, 805.

4'-hydroxy-3,4-dimethoxydihydrochalcone (207).

4'-hydroxy-3,4-dimethoxychalcone (16.85g,59.3mM) was added to 300 cc. methanol and 1g. 10% palladium/charcoal catalyst was also added.The mixture was hydrogenated at room-temperature and atmospheric pressure. Actual hydrogen uptake-3900cc.,theoretical uptake 1330cc. at S.T.P. After filtration and rotary-evaporation a grey oil was obtained which solidified after 4 hours. to yield 14.2g(83.5%) of the dihydrochalcone. m.p. 65-70°C

λ_{max} in neutral methanol 279nm., ε=5288; λ_{max} in0.1M KOH/MeOH 286nm., ε=5240. ν=3470cm.$^{-1}$ (-OH) and 1620cm.$^{-1}$ ($\rangle$C=O), 1600, 1515, 1255, 1225 (broad), 1150, 1135, 1125, 1025, 1015, 860, 825, 810, 785, 755.

The Attempted Preparation of 4'-hydroxy-3,4-dimethoxydihydrochalcone-4'-β-D-glucopyranoside(277).

4'-hydroxy-3,4-dimethoxydihydrochalcone (5g,17.5mM) was dissolved in a solution of 2% potassium hydroxide in water/acetone (1:9). α-acetobromo-glucose (7.2g,21.7mM) was added slowly during 5 minutes to the mixture.A brown,oily lower layer was formedand rotary-evaporation of this mixture yielded only a non-sweet,brown oil.No solid material could be obtained.

4'-hydroxy-3,4-methylenedioxychalcone (208).

4-hydroxyacetophenone (13.6g,100mM) and piperonal (15.0g,100mM) were added to 80cc. of 20% potassium hydroxide in water and the mixture was heated for 3 hours on a steam-bath.The mixture was poured into 300cc. water and 40cc. concentrated hydrochloric acid were added.A yellow gum resulted which was dissolved in methanol to yield 16.3g.(61%) of the recrystallised chalcone.m.p.200°C. λ_{max} in neutral methanol 354nm.,ε=24,800; λ_{max} in 0.1M potassium hydroxide in methanol 392nm.,ε=25,300;ν=3110cm.$^{-1}$ (-OH) and 1640cm.$^{-1}$ (>C=O). 1610, 1595, 1330, 1290, 1255, 1215, 1170, 1105, 1035, 1025, 985, 920, 835, 810, 800.

4'-hydroxy-3,4-methlenedioxydihydrochalcone (209).

4'-hydroxy-3,4-methylenedioxychalcone (6g,22.4mM) was dissolved in 100cc. methanol and 0.05g of 10% palladium/carbon catalyst was added.After hydrogenation at room-temperature and atmospheric pressure the mixture was filtered,rotary-evaporated and crystallised in the refrigerator overnight. 6.2g.(23%)of the dihydrochalcone was obtained.m.p. 90°C.

λ_{max} in neutral methanol 284nm.,ε=5028; λ_{max} in 0.1MKOH/MeOH 288nm., ε=7487;ν=3440cm.$^{-1}$ (-OH),shoulder at 3100cm.$^{-1}$,1620cm.$^{-1}$ (>C=O). 1600, 1245 (broad), 1175, 1115, 1100, 1065, 1025, 940, 920, 910, 900, 830, 810.

The Attempted Synthesis of 4'-hydroxy-3,4-methylenedioxydihydrochalcone-4'-β-glucopyranoside(278).

4'-hydroxy-3,4-methylenedioxydihydrochalcone (3g,11.1mM) was dissolved in a solution of 1.2g. potassium hydroxide in 100cc. of 10% water in acetone yielding a pale yellow solution. α-acetobromoglucose (4.6g,13.9mM) was added slowly over a period of 5 minutes after which the solution had changed to a dark yellow colour and a brown oil had been deposited as the lower layer. No crystalline material could be obtained following rotary-evaporation, trituration or drying in a vacuum-oven. The oil was non-sweet.

4'-hydroxychalcone(210)

4-hydroxyacetophenone (20g,147mM) was added to benzaldehyde (15.6g, 147mM) and 150cc. of 20% potassium hydroxide in water added. The mixture was heated on a steam-bath for 3 hours and the liquid was then poured onto 300g. ice. A precipitate did not form at this stage. The mixture was acidified to pH=6 whereupon a yellow precipitate resulted which quickly changed to a solid lump of gum. The gum was isolated, 200cc. of ethanol was added and the mixture was heated to boiling on a steam-bath. The gum broke up to yield a yellow solid which was collected and dried in a dessicator. Yield 22.3g(68%). m.p.168-171°C (lit.[155] 172°C). ν_{max} $cm.^{-1}$, 3200 (-OH), 1650(C O), 1610, 1570, 1345, 1290, 1230, 1180, 1050, 985, 835, 820, 760. λ =320, ε =28,100 λ =390, ε =20,800

4'-hydroxydihydrochalcone or 3-phenyl(p-hydroxy)propiophenone (211).

4'-hydroxychalcone (3g,13.4mM) was dissolved in 90cc. ethanol and 10cc. water and 0.5g. of 10% palladium/carbon catalyst was added. The mixture was hydrogenated for 1.75 hours at room temperature and atmospheric pressure. Hydrogen uptake-325cc.(theoretical uptake 300cc.). The catalyst was removed by filtration and the filtrate was rotary-evaporated to a 20cc. volume. A precipitate was not thrown down on cooling. The addition of 20cc. water caused immediate precipitation of pure white crystals. Yield 2.7g(91%). m.p.60-61°C.(lit.[156,157] 104°C, 75°C. ν_{max} $cm.^{-1}$, 3250 (-OH), 1655, 1605, 1570, 1340, 1290, 1210, 1170, 840, 745, 695. λ_{max} $cm.^{-1}$, (neut.)280 =14,500 (alk) 330 =24,800

4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetylglucopyranoside (212).

4'-hydroxydihydrochalcone (5g,22.2mM) was dissolved in a solution of 2.5g potassium hydroxide in 10% water in acetone. α-acetobromoglucose (9.1g,22.2mM) was added slowly during 5 minutes whilst stirring. A bright yellow solution changed to a brown sludge. On recrystallising once from methanol 3g.(22%) of the dihydrochalcone tetraacetylglucoside(the title compound) was obtained. A second recrystallisation was carried out. m.p. 140-5°C

;ν=1750cm.$^{-1}$ (←oAc),1680cm.$^{-1}$ (>C=O);NMR(DMSO)ppm, 8.0 doublet [2],J=8.5Hz(C_2,-H and C_6,-H);7.25 singlet [5] (aromatic protons of 'B'-ring; 7.1 doublet [2] (C_3,-H and C_5,-H);5.35 [4] (glucosyl-CH- protons at C1, C2,C3,and C4);4.2 [3] (glucosyl protons at C5 and C6);3.1 quartet [4],J=5.5Hz (Ar.CO.CH_2CH_2.Ar);2.0 [12] (4 acetyl groups -O.CO.CH_3). ν_{max} cm.$^{-1}$, 1750,1685, 1605,1586,1215(broad),1085,1065,1030,975,905,835,775,730,690.

4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetylglucopyranoside (212) using a method which employs mercuric acetate reagent.

4'-hydroxydihydrochalcone (1g,4.4mM), α-acetobromoglucose (0.69g,2.1mM) and mercuric acetate (0.4g,1.26mM) were heated together at 75°C for 30 minutes. Then 2cc. ethyl acetate and 30cc. benzene added. The solution was washed with 30cc. of 5% sodium hydroxide in water then with 30cc. water. The benzene layer was vacuum-evaporated and the residue was taken up in ethanol. A buff precipitate resultedin 0.2g. (7.4%) yield. This solid had a slightly bitter taste. m.p.140-5°C i.e. identical to that obtained by the method described in the previous section.

Hydrolysis of the 4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetylglucopyranoside (212).

The title compound (0.5g,0.9mM) was dissolved in 5cc. absolute methanol and 0.1cc. 0.1M sodium methoxide added. It was heated for two minutes on a steam-bath whilst moisture was excluded from the reaction mixture.

After rotary evaporation and storing in the refrigerator 0.16g of product was obtained.This was not the dihydrochalcone glucoside but rather the dihydrochalcone aglycone 4'-hydroxydihydrochalcone,,as is shown by the PMR spectrum.Also,the product had a m.p. of 154°C whereas 4'-hydroxydihydrochalcone has a m.p. of 60-61°C This suggests that hydrolysis took place during dissolution of the sample for the PMR spectrum determination. m.p. 154°C. The product of hydrogenation was found to be non-sweet.

NMR (DMSO) ppm, 7.85 doublet [2] J=8.5Hz (C_2,-H, C_6,-H); 7.25 singlet [5] (aromatic protons of the 'B' ring);6.9 doublet [2] J=8.5Hz (C_3,-H,C_5,-H);4.25 singlet [1] (-OH, lost on deuteration); 3.1 quartet [4] J=5.0 ($ArCOCH_2CH_2Ar$). ν_{max} $cm.^{-1}$, 1670, 1660, 1610, 1590, 1290, 1215, 1175, 980, 840, 750, 705.

Chalcone (benzylidene acetophenone) (214).

Acetophenone (100cc,85.5mM) was added to benzaldehyde (88cc,87.2mM) and 100g. potassium hydroxide was dissolved in 500cc. water.The mixture was heated on a boiling water bath for 3 hours,cooled when a dense,yellow, immiscible layer formed.which solidified to give yellow crystals.These were recovered and recrystallised from 40-60 petroleum ether.Yield 125g. (70%) m.p. 54-56°C,(authentic sample 54-56°C,lit.[158] 57-58°C) λ_{max} 310nm.,(authentic sample 312 nm.). ν =1650$cm.^{-1}$ 1660$cm.^{-1}$ 1600$cm.^{-1}$ (identical with an authentic sample). 1370, 1330, 1305, 1280, 1210, 1010, 990, 970, 855, 740, 680.

Dihydrochalcone or (3-phenylpropiophenone)(215).

Chalcone (5g,24mM) was added to 60cc. ethanol and 20cc. water and 0.5g. of 10% palladium/carbon catalyst.The mixture was hydrogenated at room temperature and atmospheric pressure.Hydrogen uptake 600cc.(theoretical uptake 540cc.).Silvery-white crystals had separated.These crystals were dissolved by heating the mixture to boiling and the catalyst was filtered off.After cooling crystals were obtained in a yield of 0.65g (13%) m.p.68-70°C (lit.[159] 72-73°C)ν_{max} 1680$cm.^{-1}$ (>C=O),1600$cm.^{-1}$ 1490$cm.^{-1}$ 1450$cm.^{-1}$ 1290, 1210, 1180, 1075, 970, 735, 695, 680.

2-hydroxyacetophenone (217).

Phenol(100g,1.06M) was acetylated by adding 100cc (2.1M) acetyl chloride,refluxing for one hour and then standing overnight.Anhydrous aluminium chloride (134g,1.01M) was next added over a period of one hour.The liquid changed in colour from yellow through brown to black.Finally,a black tar was obtained.More acetyl chloride was added to reduce its viscosity and the mixture was heated on the steam-bath for a further 30 minutes.200cc. of 50% hydrochloric acid in water was added,followed by 200cc. water.The water layer was extracted three times with benzene (3×100cc) and the benzene extract was rotary-evaporated.The pale brown residue was vacuum distilled and the fraction boilng between 100 and 110°C at ca.17mm.Hg pressure was collected. η^{21}=1.54 (lit.[158] η^{21} 1.558) Yield 10.2g.(14.1%). ν_{max} cm.$^{-1}$, 1650, 1500, 1460, 1380, 1315, 1255, 1230, 1170, 970, 760.

2'-hydroxychalcone(218).

2-hydroxyacetophenone (30.5g,0.224M) was added to benzaldehyde (23.8g,0.224M) and 150cc. 20% potassium hydroxide in water added.The mixture was heated on a steam-bath for 3 hours then poured into 200g. of ice whereupon a yellow precipitate and an orange gum resulted.The aqueous portion was decanted off and the gum and precipitate were collected,washed with water and dried.The gum was completely converted to a yellow precipitate by the addition of 500cc. of water and stirring.The solid was collected by Buchner filtration,washed with water and dried in a vacuum dessicator. Yield 35.4g.(70.5%). A melting-point of this gum was not obtained.

2'-hydroxydihydrochalcone or (3-phenyl(0-hydroxy)propiophenone.(219).

2'-hydroxychalcone (5g,22.3mM) was dissolved in 100cc. of 10% water in ethanol and 0.5g. of 10% palladium/carbon added.The mixture was hydrogenated for 4 hours at room temperature and stood overnight.Hydrogenation was restarted the next day and continued for four hours and then stood overnight again.Hydrogen uptake 250cc. (theoretical uptake 500cc.).The catalyst was filtered off and the filtrate was rotary-evaporated.On cooling, 10cc. water were added and a rather gummy precipitate was obtained.

m.p.46-50°C (lit.36-37°C).A repeat of this hydrogenation using alkaline reaction conditions did not increase the rate of hydrogenation nor was the hydrogen uptake nearer the theoretical uptake.3g. of 2'-hydroxychalcone and 0.25g. of 10% palladium/carbon catalyst were added to 20cc. water, 80cc. ethanol and 1.7g. potassium hydroxide.The mixture was hydrogenated for 3 hours at room temperature and atmospheric pressure.Hydrogen uptake 70cc. (theoretical uptake 300cc.).The mixture was stood overnight when the total uptake of hydrogen was then 140cc. The mixture was stirred for 8 hours and again stood overnight.Overall hydrogen uptake 170 cc.No attempt was made to isolate the dihydrochalcone.

Preparation of 10% Palladised Charcoal Hydrogenation Catalyst.

15g. activated charcoal were boiled for 2 hours with 10cc. concentrated hydrochloric acid and 300cc. water.The charcoal was then filtered, washed with distilled water and dried.Palladium chloride (1g.) was warmed with 1.5cc. concentrated hydrochloric acid and 10cc. water for 10 minutes. This was added to 35g. sodium acetate in 100cc. water contained in a hydrogenation flask.6g. purified charcoal was introduced and the whole mixture was hydrogenated for 16 hours. The 10% palladium/carbon catalyst was then recovered by filtration,washed with water and dried at 110°C.

A Comparison of the Catalytic Activities of Commercial 10% Pd/C Catalyst and the Catalyst Prepared by the Author.

The rate of hydrogenation of mesityl oxide ($CH_3.CO.CH=C(CH_3)_2$) was measured.Mesityl oxide (0.392g,4mM) and 300mg. 10% palladium/carbon catalyst were added to 100cc. methanol.The mixture was hydrogenated at atmospheric pressure and at room temperature (theoretical uptake 89.5cc.)The commercial catalyst reaction mixture reached the theoretical hydrogen uptake after 6 minutes hydrogenation whereas the mixture containing the catalyst prepared at Bath took 15 minutes to reach the theoretical hydrogen uptake.The Bath material was active,therefore,but not as active as the commercial material.

A Comparison of the Catalysts 10% Pd/C and Adams Catalyst(PtO_2) for Chalcone Hydrogenation.

Three chalcones were examined,Chalcone itself (i.e. benzylidene acetophenone),4'-hydroxychalcone and 2'-hydroxychalcone.To 5g. of each chalcone was added either 0.05g of Adams catalyst or 0.25g of 10% palladium/carbon catalyst and each mixture was made up to 100cc. with ethanol.The mixtures were hydrogenated at room temperature and atmospheric pressure and the results are summarised in Figure 2.

The Attempted Reduction of Several Chalcones by Use of a Zinc/Acetic Acid Reagent.

Chalcone (i.e. benzylidene acetophenone),2'-hydroxychalcone and 4'-hydroxychalcone were examined individually.To each chalcone (1g.) was added 25cc. water,25cc. acetic acid and 25cc. ethanol together with 1g. granulated zinc and these mixtures were heated on a steam-bath for one hour.For Chalcone: On cooling a white powder was precipitated which was filtered off and dried.Yield 0.065g .(2.2%) m.p. 180°C. The I.R. spectrum was identical with that obtained for Dihydrochalcone prepared by hydrogenation using 10% Pd/C catalyst.m.p. higher than expected for this compound therefore some of the solid strongly heated.A residue was absent indicating that a zinc salt was probably absent.

For 2'-hydroxychalcone: A yellow,gummy,solid mass resulted which did not become any less gummy after standing overnight in a dessicator.

For 4'-hydroxychalcone: A yellow coloured solution resulted.After addition of water 0.85g (85%) of a yellow,gummy precipitate was obtained. The filtrate was made alkaline with potassium hydroxide solution when an unidentified white solid was thrown down.The I.R. spectrum of the yellow solid was almost identical with that obtained for 4'-hydroxydihydrochalcone by hydrogenation using 10% Pd/C catalyst.

The Attempted Reduction of Several Chalcones using Diimide Reduction.

The reductions of three chalcones were examined,i.e. Chalcone itself, (i.e. benzylidene acetophenone),2'-hydroxychalcone and 4'-hydroxychalcone. 1g. of each chalcone was added to 1% copper sulphate in water (1cc.) and 50cc. ethanol.The mixture was cooled to 0°C and 6cc. of 95% hydrazine hydrate was added.Then,6cc. of 30% hydrogen peroxide was added gradually during 15minutes.Gums were obtained in every case and no crystalline material could be obtained.

3-hydroxy-4-ethoxybenzaldehyde (222).

Protocatechuic aldehyde (10g,72mM) was dissolved in 70cc. ethanol and 4.5g. of 90% potassium hydroxide in water was added,followed by 11.2g. of ethyl sulphate.The mixture was refluxed for 4 hours in a nitrogen atmosphere,then cooled and extracted with diethyl ether in order to separate thediethoxy derivative.The aqueous layer was acidified and extracted with a fresh portion of ether to extract the mono-ethoxy derivative.In fact,only the first ether extraction resulted in a mono-ethoxy compound and the diethoxy compound was not isolated,only the starting material. Yield of the title compound,3.43g.(57.1%).m.p.124-5°C (lit.[160] 127-8°C).

ν cm^{-1} 1660,1610,1580,1450,1380,1041,980,890,810,800,750.

NMR ($CDCl_3$) ppm,9.2 singlet [1] (-CHO);6.8-7.6 multiplet [3] (aromatic protons);6.1 broad singlet [1] (-OH);4.2 quartet [2] ,J=7Hz,(Ar.O.CH_2CH_3); 1.5 triplet [3] ,J=7Hz,(Ar.O.CH_2CH_3).

The Attempted Preparation of 3-hydroxy-4-(n-butoxy)benzaldehyde (224).

Protocatechuic aldehyde (10g,72mM) was dissolved in 70cc. ethanol and n-butyl bromide (10g,73mM),together with potassium hydroxide (4.5g, 80.2mM).The mixture was refluxed in a nitrogen atmosphere for 4 hours. The ethanol was evaporated off,4.5g. of potassium hydroxide was added and the solution was shaken with ether.The residue was vacuum distilled to yield water only.

The Attempted Preparation of 3-hydroxy-4-(n-propoxy)benzaldehyde (225).

Protocatechuic aldehyde (1og,72mM) was dissolved in 70cc. ethanol and n-propyl bromide (9g,73mM) was added together with 4.5g potassium hydroxide.The mixture was refluxed in a nitrogen atmosphere for 4 hours, then cooled and washed with ether(3X50cc.).The mixture was acidified and extracted with ether,then the ether was evaporated to yield tar-covered crystalswhich were dissolved in ethanol,boiled with decolorising charcoal, filtered and allowed to crystallise.7.7g. of starting material were obtained.NMR showed protocatechuic aldehyde.

The Attempted Synthesis of 4-hydroxybenzaldehyde,methylenedioxyacetal (226).

4-hydroxybenzaldehyde (10g,82mM),together with 6cc. ethylene glycol, 100cc. benzene and 0.1g. toluene p-sulphonic acid were refluxed for 12hours using a Dean-Stark water separator.A large amount of a red,gummy,jam-like material was obtained.

The Attempted Synthesis of protocatechuic aldehyde,methylenedioxyacetal(227).

Protocatechuic aldehyde (10g,72mM),together with 6cc. ethylene glycol and 0.1g. toluene p-sulphonic acid was refluxed for 12 hours using a Dean-Stark water separator.The reaction product was a black,tarry mess and no attempt was made to isolate the title compound.

4-propoxybenzoic acid(228).

4-hydroxybenzoic acid (10g,72mM),together with n-propyl bromide (9g, 73mM),8.0g. potassium hydroxide and 100cc. ethanol was refluxed under nitrogen for4 hours.The reaction mixture was cooled and acidified to yield a white precipitate which was filtered off and dried.Yield 6.1g.(47%) m.p. 140-1°C(lit.[161] 145°C). ν $cm.^{-1}$,1670,1600,1420,1240,970,840,770. NMR (DMSO) ppm;7.95 doublet [2] J=9Hz (aromatic protons);7.02 doublet [2] J=9Hz (aromatic protons);4.0 triplet [2] J=6.5 ($Ar.O.\underline{CH}_2CH_2CH_3$);1.75 quartet [2] J=7Hz ($Ar.O.CH_2\underline{CH}_2CH_3$); 1.0 triplet [3] J=7Hz ($Ar.O.CH_2CH_2\underline{CH}_3$).

The Attempted Preparation of 4-propoxybenzoyl chloride (229) &4-propoxybenzaldehyde

4-propoxybenzoic acid (5g,28mM) and thionyl chloride (25g,210mM) were refluxed for 30 minutes.0.05g. of 5% palladium on barium sulphate catalyst was added together with 50cc. xylene and hydrogen was bubbled through whilst the mixture was boiling.After a week's standing at room temperature white flaky crystals were obtained which were recrystallised from xylene. m.p. 140°C . The IR and NMR spectra were identical to those obtained for the starting material,4-propoxybenzoic acid.

The Attempted Synthesis of 4-propoxybenzaldehyde (230).

4-hydroxybenzaldehyde (50g,0.409M) was poured into 500cc. of ethanol together with n-propyl bromide (45g,0.366M) and potassium hydroxide(40g,0.714M) The mixture was refluxed in a nitrogen atmosphere for 4 hours,then cooled and acidified.Two crops of crystals were obtained which were shown to be, after acidification, 4-propoxybenzoic acid m.p. 140-2°C.ν ,$cm.^{-1}$ 1590, 1535,1380,1245,1160,1090,850,780. NMR as for 4-(n-propoxy)benzoic acid.

4-benzyloxyacetophenone (231).

4-hydroxyacetophenone (10g,73.5mM) together with benzyl chloride (9.3g, 73.5mM) and potassium hydroxide (5g,89.2mM) were dissolved in 150cc. acetone and the mixture was refluxed for 3 hours,then left overnight at room temperature.The mixture was then extracted with ether (3 100cc.) and the ether extracts were washed once with 1N potassium hydroxide in water and twice with water.The ether was evaporated to yield a cream solid which was recrystallised from benzene.The solid was dried in a dessicator.Yield 11g, (66%) m.p. 90-1°C (lit.[162] 91-2°C) ν_{max},$cm.^{-1}$ 1670,1590,1250,1000,820,750,700.

4-hydroxy-4'-benzyloxychalcone (232).

4-benzyloxyacetophenone (5g,22mM) and 4-hydroxybenzaldehyde (2.7g, 22mM) were dissolved in100cc. methanol and 2.5g. potassium hydroxide in 2cc. water were added.The mixture was refluxed for two hours on a steam-bath then left overnight at room temperature after which the mixture was neutralised.The colour changed from bright yellow (due to chalcone) to pale yellow and a white solid (KCl) precipitated.The fitrate was rotary-evaporated to yield a yellow solid which was shown by its IR spectrum to be 4-benzyloxyacetophenone (2.75g,55% of starting material).This was re-dissolved in 125cc. of 1N potassium hydroxide and extracted with ether to remove the 4-benzyloxyacetophenone.The aqueous fraction was acidified and re-extracted with ether,the ether removed by evaporation and the residue taken up in methanol.After leaving in the refrigerator over 48 hours yellow crystals of 4-hydroxybenzaldehyde were obtained.Yield 0.43g(5.9%). m.p. 112-4°C (lit.[158] 115°C). $\nu_{max}^{cm.^{-1}}$,1660,1600,1450,1280,1210,1150,850, 815,780,700. NMR ($CDCl_3$) ppm;9.95 singlet [1] (-CHO); 7.9 doublet [2] J=9Hz, (aromatic protons);7.07 doublet [2] J=9Hz (aromatic protons).

The synthesis was repeated as described above except that following acidification and ether extraction the ether layer was also washed with a solution of 20% sodium bisulphite in water in order to remove 4-hydroxybenzaldehyde starting material.After evaporation of the ether layer a white solid (4.0g.) was obtained which was shown to be,by comparison of its IR spectrum with that of 4-benzyloxyacetophenone,this latter compound.This starting material was recovered in 93% yield.

The synthesis was again repeated.4-benzyloxyacetophenone(4.0g,17.7mM) and 4-hydroxybenzaldehyde (2.2g,18mM) were dissolved in 100cc. methanol and potassium hydroxide (2g,35.7mM) was added.This mixture was left at room temperature for 3 days after which it was extracted with ether (3×100cc) to remove 4-benzyloxyacetophenone,neutralised and extracted with ether. The ether layer was washed with 10% sodium bisulphite in water (2×100cc.)

and with water alone (2 100cc.). The ether was evaporated to yield an oil. Addition of 10% water in methanol, dissolution followed by cooling yielded yellow crystals. Yield 0.65g (11%). m.p. 148-150°C. $\nu_{max}^{cm.^{-1}}$, 1660, 1625, 1600, 965, 820, 810, 720. $\lambda_{max}^{nm.}$, 350 (in neutral methanol), 424 (in 0.01N KOH in methanol). NMR (CD_3SOCD_3) ppm; 9.75 doublet [2] J=9Hz, ($C_{2'}$-H and $C_{6'}$-H); 9.2 doublet [2] J=8.5Hz, (C_2-H and C_6-H); 9.17 singlet [5] (benzylic aromatic protons); 8.87 singlet [1] (olefinic proton); 8.90 singlet [1] (olefinic proton); 8.55 doublet [2] J=9Hz ($C_{3'}$-H and $C_{5'}$-H); 8.2 doublet [2] J=8.5Hz (C_3-H and C_5-H); 6.2 singlet [2] (Ph.CH_2.O.Ar).

The Attempted Synthesis of 4-(n-propoxy)-4'-benzyloxychalcone (233).

4-hydroxy-4'-benzyloxychalcone (0.5g, 1.5mM) and n-propyl bromide (0.2g, 1.6mM) together with 0.2g potassium hydroxide were refluxed for 4 hours. The mixture was cooled, extracted with ether (2×55cc.) after dilution to 25cc. with water and the ether extracts were washed with water (2×25cc.) and then evaporated on a rotary-evaporator. No solid was obtained. The aqueous, alkaline solution was acidified and rotary-evaporated to yield a brown syrup which yielded a yellow precipitate on cooling and dilution with methanol. This solid was starting material, 4-hydroxy-4'-benzyloxychalcone (0.24g, or 48% of starting material).

4'-benzyloxybenzaldehyde (236).

4-hydroxybenzaldehyde (12g, 98mM) was added to 50cc. methanol and 4.5g. potassium hydroxide in 10cc. water. Benzyl chloride (12.5g, 98mM) was added and the mixture was refluxed for two hours on a water-bath. Rotary-evaporation yielded a pale yellow solid. Yield 14.7g. (71%). m.p. 56-76°C (lit[163] 73.5-74°C). The solid was recrystallised from methanol, m.p. 68-72°C. $\nu_{max}^{cm.^{-1}}$, 1680, 1600, 1250, 1150, 1010, 820, 720, 680.

The Attempted Preparation of 4-benzyloxy-4'-hydroxychalcone (237).

4'-benzyloxybenzaldehyde (10g,47mM) and 4-hydroxyacetophenone (6.4g, 47mM) were added to 40cc. of 20% potassium hydroxide in water and the mixture was heated for one hour on a steam-bath.After this time the mixture was poured into 150cc. water containing 20cc. hydrochloric acid .A gum resulted, which was dissolved in methanol and,after cooling, a yellow precipitate was obtained. Yield 3g. (12.4% if chalcone). 2nd. crop 4g. (16.6%) m.p.115-19 $\lambda_{max}(\varepsilon)$ nm.; in neutral ethanol,283 (20,375),345 (13,580); in 0.01M KOH/EtOH, 283 (24,904),330 (22,640).
NMR ($CDCl_3$) ppm; 7.90 doublet [2] J=8.5Hz (aromatic protons);8.95 singlet [5] (benzylic aromatic protons); 7.12 doublet [2] J=8.5 (aromatic protons); 5.2 singlet [2] ($PhCH_2$);2.6 [1] (unknown proton).The NMR spectrum does not confirm that the title compound was formed.Neither does the UV spectrum confirm that a chalcone was formed.

3-hydroxy-4-benzyloxybenzaldehyde (238).

Protocatechuic aldehyde (10g,72.5mM) was dissolved in 50cc. methanol and 3.3g. potassium hydroxide in 10cc. water was added. Benzyl chloride (9.2g,72.4mM) was poured into the mixture and the whole was heated under reflux for two hours on a steam-bath. After evaporation white crystals formed.After further cooling the crystals were collected and recrystallised from methanol. m.p. 114-116°C (lit.[164,165] 120,121-2°C). ν_{max} $cm.^{-1}$,3200, 1665,1105,1000,860,800,770,725,685.

3,4'-dihydroxy-4-benzyloxychalcone (239).

3-hydroxy-4-benzyloxybenzaldehyde (4g,17.5mM) and 4-hydroxyacetophenone (2.4g,17.6mM) were added to 20 cc. of 20% potassium hydroxide in water. the mixture was heated for 3 hours on a steam-bath, cooled and neutralised. The resulting yellow gum was recrystallised from methanol to yield 0.7g.

(11.5%) of a yellow-ochre coloured solid. m.p. $^{121-7}$ ν_{max} $cm.^{-1}$,

λ_{max} (ε)nm.; in neutral methanol,275 (12,300), 320(13,000),356 (14,100); in 0.01M KOH/MeOH 250 (31,100),285 (24,600),336 (27,500), 394 (26,800).

The Attempted Synthesis of 3,4'-dihydroxy-4-benzyloxychalcone-4'-(1-O-β-D-glucopyranoside) (240).

3,4'-dihydroxy-4-benzyloxychalcone (0.3g,0.867mM) was dissolved in a solution of potassium hydroxide (0.1g,1.8mM) in water and α-acetobromoglucose (0.3g,0.9mM) was added. The solution was allowed to stand at room temperature overnight after which time the solution was evaporated to a reduced volume. A brown oil separated but treatment of this oil with various solvents,accompanied by cooling in the refrigerator, failed to produce any solid material. When the aqueous phase(from which the oil had separated) was cooled to ca. 0°C ,only chalcone starting material was isolated.

Dihydrochalcone Glucosylation using Silver carbonate/Quinoline Reagent.

Several dihydrochalcones were examined for their possible formation of their respective glucosides. Each dihydrochalcone aglycone (3mM) was added to quinoline (15cc.) together with freshly prepared silver carbonate (3mM).The mixture was stirred as α-acetobromoglucose was added slowly during 5 minutes and then it was allowed to stand at room temperature, overnight. After filtration to remove silver oxide and rotary evaporation of the filtrate followed by cooling, only dihydrochalcone starting material was isolated in each case. Trituration of the reaction product with the common organic solvents, followed by cooling at ca.0°C failed to precipitate the dihydrochalcone glucosides.

The dihydrochalcone aglycones which were used in these attempted glucosylations are listed below:

1) 4'-hydroxydihydrochalcone. (211)

2) 4'-hydroxy-3,4-dimethoxydihydrochalcone (207)

3) 4'-hydroxy-4-methoxydihydrochalcone. (202)

4) 4'-hydroxy-3,4-methylenedioxydihydrochalcone. (209)

5) 3,4'-dihydroxy-4-methoxydihydrochalcone. (205)

This glucosylation reaction was also tried on 2,4-dihydroxyacetophenone (resacetophenone) but the glucoside was not isolated.

2',4'-dihydroxychalcones.

2',4'-dihydroxyacetophenone (resacetophenone) (50mM) was added to 40cc. of 20% potassium hydroxide in water and the aromatic aldehyde (50mM) added. The mixture was heated for 3 hours on a steam-bath, then cooled and neutralised. All the reactions tried gave brown, gummy residues except the reaction of 3,4-dimethoxybenzaldehyde with resacetophenone to yield a yellow precipitate of 2',4'-dihydroxy-3,4-dimethoxychalcone (247). Yield 5.3g. (34%). m.p. 198-202°C ν_{max} cm.$^{-1}$, 3200, 1630, 1145, 1030, 1010, 975, 965, 840, 810, 785. λ_{max} (ε) nm., in neutral ethanol, 260 (11,200), 305sh., 376 (28,600); in 0.01M KOH/EtOH, 255sh., 284sh., 335 (32,000), 402 (40,400).

The substituted benzaldehydes which were contacted with resacetophenone but which did not yield chalcones were as follows: benzaldehyde, 4-hydroxybenzaldehyde, 4-methoxybenzaldehyde (anisaldehyde), 3-hydroxy-4-methoxybenzaldehyde (isovanillin) and 3,4-methylenedioxybenzaldehyde (piperonal). Therefore, the following chalcones were not isolated:

1) 2',4'-dihydroxychalcone. (248)

2) 2',4,4'-trihydroxychalcone. (249)

3) 2',4'-dihydroxy-4-methoxychalcone. (250)

4) 2',3,4'-trihydroxy-4-methoxychalcone. (251)

5) 2',4'-dihydroxy-3,4-methylenedioxychalcone. (252)

The Attempted Preparation of 2',4'-dihydroxy-3,4-dimethoxydihydrochalcone(252a)

2',4'-dihydroxy-3,4-dimethoxychalcone (5g,16mM) was dissolved in 100cc. methanol and 0.5g. of 10% palladium/carbon catalyst was added. The mixture was hydrogenated at room temperature and atmospheric pressure for 12 hours, then it was filtered and rotary-evaporated to yield a brown oil.The dihydrochalcone was not isolated following cooling and trituration procedures.

The Preparation of Dihydrochalcone Glucosides from Tetraacetylpicein.

2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide. (α-acetobromoglucose) (153).

α-acetobromoglucose was prepared by the method described in 'Organic Syntheses'[166] by which D-glucose is acetylated with acetic anhydride after which hydrogen bromide gas is bubbled into the solution. Yields obtained on subsequent syntheses were: 39,30,43,36,25,51,60% .m.p. 88°C (lit.[168] 88-89°C). $\nu_{max}^{cm.^{-1}}$, 1740,1370,1230,1110,1040,920,910,890,840,750.

4-hydroxyacetophenone-4-(2,3,4,6-tetraacetyl-β-D-glucopyranoside). or tetraacetylpicein.(253).

Acetobromoglucose (55g,0.134M) in 300cc. acetone was added to a solution of 4-hydroxyacetophenone (19g,0.140M) and potassium hydroxide (8.5g,0.150M) in 300cc. distilled water and 100cc. acetone. The mixture was left overnight at room temperature, then rotary-evaporated to a volume of 400cc. when a bulky, cream-coloured solid was precipitated. The mixture was left in a refrigerator overnight, then filtered, washed with 100cc. water and dried in a vacuum dessicator. Yield 23.2g. (37%). Yields on subsequent syntheses, 18,24,37,25,38%. m.p. 166-7°C (lit.[167,68] 172-3°C). $\nu_{max}^{cm.^{-1}}$, 1740,1670, 1600,1365,1220,975,960,890,830. NMR ($CDCl_3$) ppm, 8.0 doublet [2] J=9Hz, (C_2-H and C_6-H); 7.10 doublet [2] J=9Hz (C_3-H and C_5-H); 5.3 singlet [5], (Ac.O. CH); 4.3 multiplet [2] J=2Hz (R.CH.CH_2OAc); 2.6 singlet [3], (Ar.CO.CH_3);2.08 singlet [12] (-O.CO.CH_3)

OR

The solubility of tetraacetylpicein in several solvents was investigated. This compound was highly soluble in benzene,chloroform,methylene dichloride

and acetone; soluble in xylene and toluene but insoluble, both hot and cold, in 60:40 petroleum ether, diethyl ether and di-isopropyl ether. In methanol or ethanol tetraacetylpicein was relatively insoluble in the cold solvent but highly soluble in the hot solvent. These two solvents were, therefore,the only suitable recrystallisation solvents found.

Tetraacetylpicein Synthesis using Silver carbonate Reagent

α-acetobromoglucose (2g,5mM) together with 4-hydroxyacetophenone (0.67g,5mM) was dissolved in sodium-dried methylene dichloride. Silver carbonate (1.4g,5mM) was added and the mixture was stirred overnight,after which it was filtered to remove the silver oxide, rotary-evaporated and cooled. Unchanged α-acetobromoglucose was recovered and tetraacetylpicein was not isolated.

4'-hydroxychalcone-4'-(1-O-β-D-glucopyranoside) (254).

Tetraacetylpicein (2g,4.3mM) together with benzaldehyde (0.6g,5.2mM) was dissolved in 10cc. methanol and 40cc. of 10M potassium hydroxide in water was added. The mixture was allowed to stand at room temperature for 6 days (heating on a steam-bath resulted in the formation of yellow gums). The pale yellow solution was neutralised with 6M hydrochloric acid. Yield of pale yellow solid, 0.8g. (32.3%). m.p. 178-82°C (lit.[72] 195°C) ν_{max} $cm.^{-1}$, 3400 (~OH), 1650 (>C=O), 1600, 1400, 1220, 1160, 1065, 830, 699. λ_{max} (ε) nm. in neutral ethanol, 282 (12,800); in 0.01M KOH/EtOH 335 (13,600). NMR (DMSO) ppm., 7.3 complex multiplet [11] (9 aromatic protons, 2 olefinic protons); 4.3 singlet [4] (OH,removed by deuteration); 3.5 complex multiplet [6] (methylene and methine protons of glucose); 1.67 singlet [1] (C_5-H) of glucose).

The Attempted Synthesis of 4'-hydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (255). from tetraacetylpicein.

4'-hydroxychalcone-4'-(1-O-β-D-glucopyranoside) (0.2g,0.78mM) was dissolved in 10cc. methanol and 0.05g. of 10% palladium/carbon catalyst added.The mixture was hydrogenated overnight at room temperature and atmospheric pressure to yield a pale yellow liquid. Rotary evaporation yielded a pale yellow gum which could not be crystallised from the common organic solvents. This gum was non-sweet. ν max $cm.^{-1}$, 3400, 1655, 1600, 1510,1405, 1230, 1165, 1070, 1040 (sh), 835, 750, 695.

4,4'-dihydroxychalcone-4'-(1-O-β-D-glucopyranoside) (257).

Tetraacetylpicein (1.5g,3.2mM) and 4-hydroxybenzaldehyde (0.8g,6.6mM) were added to 40cc. methanol and 0.5g. sodium hydroxide in water added. There was no instant development of the yellow colour which is characteristic of chalcone formation. Needle-like, colourless crystals separated, m.p. 184-190°C (m.p.picein,lit. 192-194°C). ν_{max} $cm.^{-1}$, 3350 (-OH), 1660 (>C=O), 1605, 1580, 1285, 1245, 1185, 1085, 1035, 990, 960, 895, 845, 835. NMR (DMSO) ppm.,8.0 doublet [2] J=9Hz (C_2-H and C_6-H); 7.2 doublet [2] J=9Hz (C_3-H and C_5-H); 4.5 broad multiplet [5] (glucosyl methine protons); 3.65 broad multiplet [2] (R.CH(OH).CH_2OH); 3.3 broad singlet [4] (glucosyl-OH); 2.55 singlet [3] (Ar.CO.CH_3).

The preparation was repeated. Tetraacetylpicein (2g,4.3mM) and 4-hydroxybenzaldehyde (0.6g,5.4mM) was dissolved in 10cc. of methanol. 40cc. of a 10M solution of potassium hydroxide in water was added when the initial colour of the solution was orange. After standing at room temperature for 6 days the colour of the solution was dark yellow. The solution was cooled and acidified to pH=7 to yield a yellow gummy solid. Yield 0.4g. (17%). It was not possible to obtain a m.p. on this gum-like material. ν_{max} $cm.^{-1}$, 3400 (-OH), 1660 (>C=O), 1600, 1280, 1160, 1075, 1030, 895, 830, 785, 720. NMR (DMSO) ppm. 9.4 complex multiplet [4] (aromatic protons); 8.2 complex multiplet [6] (aromatic protons and 2 olefinic protons);

7.0 broad singlet [5] (4 glycosidic -OH, one phenolic -OH, all removed by deuteration); 4.0 multiplet [6] (methylene and methine glycosyl protons); 2.1 singlet [1] (C_5-H of glucose).

The preparation was repeated, Tetraacetylpicein (5.8g,12.4mM) and 4-hydroxybenzaldehyde (1.5g,12.3mM) was dissolved in 10cc. methanol and 80cc. of 10M potassium hydroxide in water was added. The mixture was allowed to stand at room temperature for 5 days, then neutralised to pH=7 with 6M hydrochloric acid. Initially, a fine yellow precipitate resulted but as neutralisation proceeded it changed to a dark yellow-brown gum adhering to the sides of the beaker. On standing at 0°C a yellow-brown gummy precipitate resulted. This material was hydrogenated directly but no crystalline material could be isolated.

The Attempted Synthesis of 4,4'-dihydroxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (258).

Although a crystalline 4,4'-dihydroxychalcone glucoside could not be isolated, the yellow-brown gummy precipitate described in the last section was hydrogenated overnight at room temperature and at atmospheric pressure using 0.1g. of 10% palladium/carbon catalyst. After filtration and rotary-evaporation of the filtrate no crystalline material could be isolated. The gummy residue was not sweet NMR (DMSO) ppm. 7.5 to 6.6 complex multiplet (aromatic H); 4.9 singlet (glucosyl H); 3.5 singlet ($-CH_2CH_2-$).

4'-hydroxy-4-methoxychalcone-4'-(1-O-β-D-glucopyranoside) (259).

Tetraacetylpicein (0.5g,1.1mM) and 4-methoxybenzaldehyde (anisaldehyde) were dissolved in 5cc. of methanol. 10cc. of 10M potassium hydroxide was added when there was an instant precipitation of white needles, which slowly turned yellow. The mixture was left standing at room temperature for 3 days after which time some of the pale yellow precipitate still remained. The mixture was neutralised to pH=7 and the pale yellow precipitate was collected by Buchner filtration, washed with water and recrystal-

lised from 50:50 methanol/water. Yield 0.13g. (29%). m.p. 145-8°C. ν_{max} cm.$^{-1}$, 3150 (-OH), 1640 (>C=O); 1600, 1590, 1280, 1215, 1160, 1030, 965, 820. $\lambda_{max}(\varepsilon)$ nm., in neutral ethanol, 342 (26,150), in 0.01M KOH/EtOH 342 (41,100).

4'-hydroxy-4-methoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (260).

4'-hydroxy-4-methoxychalcone glucoside (259) was added to 20cc. methanol together with 0.1g. of 10% palladium/carbon catalyst. The mixture was hydrogenated overnight at room temperature and at atmospheric pressure, filtered and the filtrate rotary-evaporated to yield 0.63g. (62.7%) of a pale yellow, non-sweet, wax-like solid. m.p. 78-80°C. ν_{max} cm.$^{-1}$, 3200 (-OH), 1660 (>C=O), 1620,1510, 1075, 1030, 940, 925, 830. λ_{max} (ε) nm., in neutral ethanol 266 (15,400); in 0.01M KOH/EtOH 266 (14,400). NMR (DMSO) ppm. 7.95 doublet [2] J=8Hz (C_2,-H and C_6,-H); 7.2 doublet [2] J=9Hz (C_2-H and C_6-H); 7.1 doublet [2] J=8Hz (C_3,-H and C_5,-H); 6.8 doublet [2] J=9Hz (C_3-H and C_5-H); 5.0 broad singlet [1] (a glycosyl proton); 5.4 singlet [4] (-OH, removed by deuteration); 4.45 singlet [3] (-O.CH_3); 4.0 to 2.5 broad multiplet [7] (glycosyl protons).

3,4'-dihydroxy-4-methoxychalcone-4'-(1-O-β-D-glucopyranoside) (261).

Tetraacetylpicein (2g,4.3mM) and 3-hydroxy-4-methoxybenzaldehyde (isovanillin) (0.75g,5mM) were dissolved in 10cc. methanol by warming to 40°C for 5 minutes. After cooling to room temperature 40cc. of 10M potassium hydroxide in water were added and the mixture was allowed to stand for 6 days. Yield 1.16g,(45%). m.p. 181-185°C. Subsequent preparations gave yields of 7.7% (m.p. 180-184°C) and 1.4% (no m.p. because a gummy solid had resulted). ν_{max} cm.$^{-1}$, 3350 (-OH), 1640 (>C=O), 1260, 1200, 1150,1115,

1030, 970, 830, 790, 780, 750, 710. λ_{max} (ε) nm., in neutral ethanol 360 (33,600), in 0.01M KOH/EtOH 400 (47,500). NMR (DMSO) ppm., 8.0 to 6.0 complex multiplet [9] (7 aromatic protons, 2 olefinic protons); 6.7 broad singlet [5] (-OH of glucose and a phenolic-OH, all removed by deuteration); 5.0 to 3.5 broad multiplet [9] (methylene and methine protons of glucose and $-O.CH_3$); 2.1 singlet [1] (C_5-H of glucose).

3.4'-dihydroxy-4-methoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (262).

3,4'-dihydroxy-4-methoxychalcone-4'-glucoside (0.5g, 1.15mM) was added to 20 cc. methanol together with 0.1g. of 10% palladium/carbon catalyst. The mixture was hydrogenated overnight at room temperature and at atmospheric pressure, filtered and the filtrate rotary-evaporated to yield a pale brown gum which was non-sweet. ν_{max} $cm.^{-1}$, 3400, 1660, 1600, 1510, 1440, 1245, 1165, 1130, 1075, 1020, 840, 800, 760. NMR (DMSO) ppm. 7.3 to 6.2 broad singlet (aromatic protons); 4.75 singlet (glucosyl protons); 3.5 singlet ($-CH_2CH_2-$). ν_{max} $cm.^{-1}$, 3400, 1660, 1600, 1510, 1440, 1245, 1165, 1130,1075, 1020, 840, 800, 760.

4'-hydroxy-3,4-dimethoxychalcone-4'-(1-O-β-D- glucopyranoside) (263).

Tetraacetylpicein (3g,6.4mM) and 3,4-dimethoxybenzaldehyde (1.1g,6.6mM) were added to 80cc. of 30% potassium hydroxide in water and the mixture was allowed to stand at room temperature for 6 days. Then, the solution was acidified to pH=7 whereupon a bright yellow gummy solid resulted. Yield 0.11g. (3.8%). m.p. 180-2°C. ν_{max} $cm.^{-1}$, 3150 (-OH), 1640 (>C=O), 1600,1370, 1260, 1210, 1150, 1030, 1015, 825, 800.

λ_{max} (ε) nm., in neutral ethanol 356 (20,800), in 0.01M KOH/EtOH 392 (21,400).

4'-hydroxy-3,4-dimethoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (264).

4'-hydroxy-3,4-dimethoxychalcone-4'-glucoside ([263]) (0.11g,0.24mM) was added to 20cc. methanol together with 0.1g. of 10% palladium/carbon catalyst. The mixture was hydrogenated overnight at room temperature and at atmospheric pressure, filtered and the filtrate rotary-evaporated to yield a pale brown gum which was non-sweet.

NMR (DMSO) ppm. 7.2 to 6.2 broad singlet (aromatic protons); 4.5 singlet (glucosyl protons); 3.5 singlet ($-CH_2CH_2$).

4'-hydroxy-3,4-methylenedioxychalcone-4'-(1-O-β-D-glucopyranoside) (265).

Tetraacetylpicein (4g,8.6mM) and 3,4-methylenedioxybenzaldehyde (piperonal) were added to 80cc. of 30% potassium hydroxide in water and the mixture was allowed to stand for 6 days at room temperature. A copious yellow precipitate had formed. The mixture was acidified to pH=7 and the solid, which was crystalline and not gummy, was collected, washed with water, dried in air and recrystallised from methanol. Yield 2.2g (60%)

m.p. 166-8°C. A subsequent synthesis gave a yield of 48%, m.p. 172 and 175-180°C. ν_{max} $cm.^{-1}$, 3350 (-OH), 1650 ( )C=O), 1600, 1255, 1070, 1030, 925, 795. λ_{max} (ε) nm.,356 (26,300) in neutral ethanol.
NMR (DMSO) ppm., 7.75 doublet [2] J=9Hz (C_1,-H and C_6,-H); 7.25 split doublet [2] J=6Hz, J=2Hz (C_6-H and C_2-H buried); 6.75 doublet [2] J=9Hz (C_3,-H and C_5,-H); 6.5 doublet [1] J=9Hz (C_5-HO; 5.65 singlet [2] (-O.CH_2.O-); 4.6 broad singlet [1] (glycosyl proton); 3.65 broad singlet [4] (-OH, removed by deuteration); 4.0 to 2.5 broad multiplet [7] (glycosyl protons).

4'-hydroxy-3,4-methylenedioxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (266)

4'-hydroxy-3,4-methylenedioxychalcone glucoside (265) (1.0g,2.32mM) was added to 20cc. of methanol together with 0.1g. of 10% palladium/carbon catalyst. The mixture was hydrogenated overnight at room temperature and at atmospheric pressure, filtered and the filtrate rotary-evaporated to yield 0.72g. (71.8%) of a non-sweet, white solid which was recrystallised from methanol. m.p. 115-118°C. λ_{max} (ε) nm., 267 (16,800), 268(19,500) in neutral ethanol.
NMR (DMSO) ppm., 7.95 doublet [2] J=8.5Hz (C_2,-H and C_6,-H); 7.1 doublet [2] J=8.5Hz (C_3,-H and C_5,-H); 6.84 singlet [1] (C_2-H); 6.72 singlet [2] (C_5-H and C_6-H); 5.9 singlet [2] (-O.CH_2.O-); 4.55 singlet [4] (-OH, removed by deuteration); 4.0 to 2.5 broad multiplet [7] (glycosyl protons).

In a another attempt at the preparation of the title compound tetra-acetyl picein (24g,51.6mM) was dissolved in 160cc. acetone and 6.6g. potassium hydroxide in 120cc. water added. The mixture became syrupy so this was extracted into methylene dichloride and evaporated to a syrup. Piperonal (7.74g,51.6mM) was added, together with 10g. potassium hydroxide. On cooling, yellow needles precipitated. m.p. 100-104°C (m.p. of the chalcone, compound 175-180°C). The material was hydrogenated. Both the hydrogenated compound and the compound of m.p. 100-104°C were shown to be the result of (see compounds 267 & 268).

the condensation of acetone with piperonal. m.p. 100-104°C . ν_{max} cm.$^{-1}$, 1660,1620,1600. λ_{max} nm. 342,sh. 300. NMR (DMSO) ppm. 7.5 doublet [1] J=10.6, (Ar.CH=CH.CO.CH$_3$); 7.3 doublet [1] J=6Hz (C_6-H); 7.1 doublet [1] J=2Hz (C_2-H); 6.9 doublet [1] J=8.5Hz (C_5-H); 6.65 doublet [1] J=10.6Hz, (Ar.CH=CH.CO.CH$_3$); 6.05 singlet [2] (-O.CH$_2$.O-); 2.3 singlet [3] (Ar.CH=CH.CO.CH$_3$). ν_{max} cm.$^{-1}$, 3350, 1660, 1655, 1620, 1600, 1485, 1440, 1235, 1165, 1065, 1030, 920, 830, 805.

Solubility Tests on several Chalcone Glucosides.

The chalcone glucosides described above were all found to be insoluble in hot or cold chloroform, methylene dichloride, diethyl ether and acetone. They were soluble in cold dimethylsulphoxide. These chalcones were insoluble in cold water and formed gums when warmed in water. The only suitable recrystallisation solvents found were ethanol and methanol- the chalcones being slightly soluble in the cold and soluble in the hot solvent.

2-methoxy-4'-hydroxychalcone-4'-(1-O-β-D-glucopyranoside).(269).

Tetraacetylpicein (0.5g,1.07mM) and 2-methoxybenzaldehyde (0.2g,1.5mM) were dissolved in 5cc. methanol. 10cc. of 10M potassium hydroxide in water were added and the mixture was allowed to stand at room temperature for 3 days. The mixture was neutralised, then left overnight and the yellow precipitate was recovered and recrystallised from 50:50 methanol/ water. Yield 0.12g. (19%). m.p. 178-180°C. Subsequent preparation, yield 45%. ν_{max} cm.$^{-1}$, 3400(-OH), 1640(>C=O), 1330, 1240, 1160, 1070, 1020, 830, 750. λ_{max}(ε)nm., in neutral ethanol 343 (32,500); in 0.01M KOH/EtOH 386 (37,800). NMR (DMSO) ppm., 8.0 to 6.0 complex multiplet [10] (aromatic and olefinic protons); 4.8 broad singlet [4] (-OH, removed by deuteration); 4.05 singlet [3] (Ar.O.CH$_3$); 3.5 to 2.5 broad multiplet [7] (glycosidic protons).

2-methoxy-4'-hydroxydihydrochalcone-4'-(1-O-β D-glucopyranoside)

Brown gum obtained by hydrogenation of chalcone (269). ν_{max} cm.$^{-1}$, 3350, 1650, 1595, 1510, 1485, 1455, 1435, 1230, 1165, 1105, 1065, 1030, 830, 745.

Phloroacetophenone (273).

Phloroglucinol (25.2g,0.2M) and anhydrous acetonitrile (16.4g,0.4M) were added to 100cc. of sodium-dried diethyl ether in a 300cc. round-bottomed flask. Finely powdered, fused zinc chloride was added (5g,37mM), the flask cooled in an ice/salt mixture and hydrogen chloride gas passed through the mixture for two hours. The mixture was then allowed to stand overnight in the refigerator and then hydrogen chloride gas was passed for another two hours into the cooled mixture. After standing for 3 days in the refrigerator a precipitate of the ketimine hydrochloride had formed. This was transferred to a beaker containing one litre of water and the whole was boiled vigorously for two hours, 5g. decolourising carbon added, boiled for another 5 minutes, filtered and allowed to stand overnight. Cream-coloured needles had formed which were collected and dried at 120°C. Yield 29g. (75%). m.p. 217-219°C (lit[168] 219°C). A repeat preparation gave a 33% yield. ν_{max} cm.$^{-1}$, 3200 (-OH), 1650 (>C=O), 1390, 1300, 1180, 1075, 970, 835, 820, 765. NMR ($CD_3.CO.CD_3$) ppm.,6.0 singlet [2] (aromatic protons); 3.5 broad singlet [3] (-OH, removed by deuteration); 3.15 singlet [3] ($Ar.CO.\underline{CH}_3$).

Phloroacetophenone-4'-(1-O-β-D-glucopyranoside) (137).

Phloroacetophenone (13g,77.4mM) and α-acetobromoglucose (36g,87.6mM) were dissolved in 75cc. acetone. 40cc. of 10% potassium hydroxide in water were added and the mixture was left standing for 4 days. The mixture was evaporated under vacuum,at a bath temperature not greater than 55°C to a red-brown syrup. The mixture was left in a refrigerator for two hours after which a copious pink solid had precipitated. This was collected, recrystallised from water once and dried at 50°C in a vacuum-oven. Yield 32.3g. (72%). m.p. 84°C. (lit[149,150] 215-16°C, 218-19°C). NMR (DMSO) ppm., 8.0 to 6.0 broad singlet [2] (-OH,removed by deuteration); 5.82 singlet [2] ($C_3-\underline{H}$ and $C_5-\underline{H}$); 5.5 to 3.5 broad multiplet [4] (glycosidic protons); 2.55 singlet [3] ($ArCO\underline{CH}_3$);

2.2 singlet [1] (C_5-H of glucose); 2.0 singlet [12] (O.CO.CH_3).

Subsequent attempts to prepare this glucoside failed. It is likely that the NMR spectrum outlined above was obtained on a mixture of phloroacetophenone and 2,3,4,6-tetraacetylglucopyranose. The latter compound was also isolated in the attempted synthesis of 3,4'-dihydroxy-4-methoxy-4'-(1-O-β-D-glucopyranoside) by reaction of α-acetobromoglucose with 3,4'-dihydroxy-4-methoxydihydrochalcone. The IR spectrum obtained was as follows: $\nu_{max}^{cm.^{-1}}$, 1750(-OAc), 1375, 1230, 1135, 1070, 1030, 980, 945, 930, 910, 900, 840, 740. m.p. 102-4°C before recrystallisation, 128-131°C after recrystallisation. (lit.[158] m.p. for 2,3,4,6-tetraacetyl-β-D-glucopyranose 132-4°C.

A Comparison of several Methods for the Preparation of Phloroacetophenone and Resacetophenone Glucosides.

A) Phloroacetophenone glucoside.

Method 1, Zemplen's method with potassium hydroxide.

Phloroacetophenone (4.9g,29.2mM) and α-acetobromoglucose (12g,29.2mM) were dissolved in 100cc. acetone. Potassium hydroxide (1.7g,30.4mM) in 10cc. water were added and the mixture was allowed to stand at room temperature overnight. The mixture was evaporated under vacuum to a cream, gummy solid. This was dissolved in 30cc. methanol and left for two days in the refrigerator-no crystals resulted. TLC showed only phloroacetophenone starting-material.

Method 2,Zemplen's method with silver carbonate.

Phloroacetophenone (4.9g,29.2mM) and α-acetobromoglucose (12.0g,29.2mM) together with silver carbonate (6g,21.8mM) in 100cc. acetone were stirred at room temperature overnight.Then the silver oxide was filtered off and the filtrate was evaporated under vacuum to a brown gum. This was dissolved in 30cc. methanol and stored in a refrigerator for 3 days. No crystals formed. TLC showed 2 spots-one of phloroacetophenone, the second probably the glucoside.R_f,0.75. Plates, silica gel; solvent, 4:1:5 n-BuOH/HOAc/H_2O .

B) Resacetophenone glucoside.

Method 1, Zemplen's method with potassium hydroxide.

Resacetophenone (4.4g,29mM) and α-acetobromoglucose (12g,29.2mM) were dissolved in 100cc. acetone. Potassium hydroxide(1.7g,30.4mM) in 10cc. water was added and the mixture was left overnight at room temperature. A brown syrup formed which was taken up in methanol and stored in the refrigerator for 3 days. No crystals formed. The solution was evaporated under vacuum and stored in a refrigerator overnight. Again, no crystals had formed. TLC showed only a tiny spot of R_f 0.75. Plates, silica gel; solvent, 4:1:5 n-BuOH/HOAc/H_2O.

Method 2, Zemplen's method with silver carbonate.

Resacetophenone (4.4g,29mM) and α-acetobromoglucose (12g,29.2mM) together with silver carbonate (6g,21.8mM) in 100cc. acetone were stirred at room temperature overnight. The silver oxide was filtered off and the filtrate evaporated under vacuum to yield a brown gum. This was dissolved in 30cc. methanol and stored in a refrigerator for 3 days. No crystals formed. After a second evaporation and cooling no crystals were isolated. TLC showed 2 spots-one of resacetophenone, the second being, probably, resacetophenone glucoside R_f 0.75. TLC method , plates, silica gel; solvent 4:1:5 n-BuOH/HOAc/H_2O.

The Synergistic Increase in Sweetness produced by Dihydrochalcones with Several Sweeteners.

Five dihydrochalcones were examined in order to determine whether or not they produce a synergistic increase in sweetness when added to several sweeteners. These dihydrochalcones were: 4'-hydroxydihydrochalcone (211), 4'-hydroxy-4-methoxydihydrochalcone (202), 4'-hydroxy-3,4-methylenedioxydihydrochalcone (209), 3,4'-dihydroxy-4-methoxydihydrochalcone (205), 4'-hydroxy-3,4-dimethoxydihydrochalcone (207).

The dihydrochalcone was added to a 5% solution of glucose, sucrose sorbitol or mannitol so that the solution was 0.05M with respect to the dihydrochalcone. The solutions were warmed but the dihydrochalcones did not readily dissolve in the aqueous media and the solutions were, therefore, cooled and filtered in order to remove the oily droplets of dihydrochalcone. The sweetnesses of the sweetener solutions alone and together with the various dihydrochalcones were assessed by the author. These observations together with comments on side-effects appear in Table 8. 3,4'-dihydroxy-4-methoxydihydrochalcone produced an enhanced sweetness sensation when added to 5% solutions of glucose, sorbitol and mannitol. It produced a simultaneous burning sensation on the tongue. The other four dihydrochalcones produced no sweetness enhancement.

PART C

THE SYNTHESIS OF

DIHYDROCHALCONE DISACCHARIDES

D I S C U S S I O N

The Preparation of Naringin and Neohesperidin Dihydrochalcones For Sweetness Evaluation

The purpose of the work described in this section was to prepare naringin dihydrochalcone (80) and neohesperidin dihydrochalcone (81) to assess the sweetness characteristics of these two sweet compounds and to measure their sweetness intensities.

Naringin flavanone (3) was obtained by sieving the deposit from twelve barrels of 2:1 grapefruit base. Better still, this compound was purchased from Sunkist. Naringin chalcone (8) was prepared by treating naringin flavanone with alkali and the chalcone was hydrogenated to naringin dihydrochalcone (80). Some data covering hydrogen yields are shown in Table 9

In order to prepare neohesperidin dihydrochalcone (81), two methods were tried, the first being the method of Krbechek[4] in which phloroacetophenone-4'-β-neohesperidoside (84) is formed from naringin by alkaline hydrolysis (see page 21). The phloroacetophenone-4'-β neohesperidoside (84) is then condensed with isovanillin to form neohesperidin chalcone which is hydrogenated to form neohesperidin dihydrochalcone (81). A yield of 35% was obtained by this method. It is worth noting that after the first preparation of phloroacetophenone-4'-β-neohesperidoside, the spot produced by this compound during thin-layer chromatography on silica gel was confused with the spot due to naringin flavanone, until it was realised that the spot due to the phloroacetophenone compound (84) was not fluorescent, whereas that due to naringin flavanone did exhibit fluroescence.

It was thought at first, that the 4-hydroxybenzaldehyde produced by the alkaline hydrolysis of naringin was recombining with the phloroacetophenone compound (84) to reform the parent naringin. This conjecture was invalidated by observation of the course of the alkaline hydrolysis of naringin by the withdrawal of samples and the examination of their U.V. spectra. The chalcone absorbance at 440 nm. diminished as the heating proceeded until, after two hours, no further diminution in the absorption occurred and the reaction was judged complete.
A rapid test which may be undertaken on a solid whose identity is either phloroacetophenone-4'-β-neohesperidoside or naringin flavanone is to record its U.V. spectrum and to observe the wavelength shift of the major peak on making the solution alakaline. The phloroacetophenone compound shifts from 285 to 295 nm. whereas, naringin flavanone shifts from 285 nm. to 430 nm. (see Spectrum 7 and 8).

In connection with Krbechek's method for neohesperidin dihydrochalcone synthesis, this author states that he prepared neohesperidin chalcone and then heated the chalcone at 80 -90°C in water for 30 minutes to yield a precipitate of neohesperidin flavanone. The precipitate was collected at 40°C and the crude neohesperidin was triturated with water at 65°C and filtered at this temperature to yield neohesperidin free of any phloroacetophenone-4'-β-neohesperidoside. It is not clear from this description whether or not it was the neohesperidin or the phloroacetophenone compound which was the most insoluble. Since it was the neohesperidin which was triturated, one might infer

that this material was the more insoluble. To resolve this problem the solubilities of naringin flavanone and phloroacetophenone-4'-β-neohesperidoside were determined (see page 141) by the author. A mean extinction coefficient of 18, 825 ± 205 @ 285 nm. was found experimentally for the phloroacetophenone compound whilst for naringin flavanone ε mean was 17, 875 ± 305 @ 285 nm. For the phloroacetophenone compound the solubilities in water at 21°C and 63.5°C was 0.90 7.8°g. l^{-1} For naringin flavanone the solubilities in water at 21°C and 63.5°C were 12.0 and 91.0 g.l^{-1} (see Table 9). The solubility of naringin flavanone was checked by a bulb technique (see page 142) when the solubilities found were 75.4 ± 5.2 g.e^{-1} at 63.5°C for naringin flavanone in water. Although a solubilitiy measurement for neohesperidin flavanone has not been obtained, the above solubility results suggest that the flavanone material is the more soluble material and that trituration methods, are unlikely to be effective as a means of purification.

The effect of alkali concentration on the yield of neohesperidin chalcone was determined by adding between 5% w/v and 50% w/v solutions of potassium hydroxide in water to weighed quantities of phloroaceto-phenone-4'-β-neohesperidoside and isovanillin. The solutions were refluxed for ten minutes in each case and after this treatment the solutions were cooled and their absorptions were read at 450 nm. (see Table 10). As the alkali concentration was increased the absorption at 450 nm also increased, so that the maximum chalcone absorption was obtained when ca. 50% w/v.

potassium hydroxide in water was used.

The effect of the duration of refluxation on the yield of chalcone was also ascertained. The result was that a one-hour refluxation of phloroacetophenone-4'-β-neohesperidoside and isovanillin in 25% w/v potassium hydroxide in water did not result in a higher yield of neohesperidin chalcone than a ten-minute refluxation.

The second method of neohesperidin dihydrochalcone preparation was that of Horowitz[49] in which naringin flavanone and isovanillin were dissolved in alkali and the mixture was refluxed to form a mixture of naringin chalcone and neohesperidin chalcone with the neohesperidin compound predominating, because a two-fold excess of isovanillin was used. The mixture was hydrogenated directly to yield neohesperidin dihydrochalcone (81) in a yield of 3%.

The yields of naringin dihydrochalcone and neohesperidin dihydrochalcone obtained by the author were sometimes very low i.e. 12% for naringin dihydrochalcone (lit 25 - 95%) and for neohesperidin dihydrochalcone - 35% by the Krbechek method and 3% by the direct method of Horowitz (lit 33 - 34%). These low yields may be attributed to one or more of the following causes :-

a) In the case of naringin dihydrochalcone, the incomplete conversion of naringin flavanone to naringin chalcone.

b) In the case of neohesperidin dihydrochalcone the incomplete conversion of phloroacephenone-4'-β-neohesperidoside to neohesperidin chalcone.

c) The reversion of chalcone to flavanone during hydrogenation in neutral media.

d) The incomplete hydrogenation of the chalcone.

e) The formation of undesired side-products.

f) The difficulty in isolating the dihydrochalcone from the hydrogenation mixture.

It is possible that low yields of naringin dihydrochalcone were sometimes obtained because the hydrogenation of naringin chalcone was carried out in neutral solution and the chalcone may have reverted back to the flavanone to some extent. Horowitz obtained a yield of 95% in neutral solution by this method[48] whereas Feldman [137] obtained a yield of 25 to 27% when he carried out the reaction in neutral solution and 72% in a solution of aqueous ethanol containing 1% potassium hydroxide. It is unlikely that incomplete hydrogenation is responsible for low yields because the actual uptake of hydrogen can be monitored and it is usually approximately equal to the theoretical uptake expected. It is likely that undesired side products were formed during the evaporation procedure. After hydrogenation the colour of the filtered, hydrogenated mixtures were pale yellow, whereas, following an approximately twenty-hour rotary evaporation under reduced pressure a dark brown oil was obtained. A more rapid evaporation would probably reduce the amount of side products and therefore improve the yield.

The final stage of crystallising the dihydrochalcones from an aqueous or acetone solution is the step in which the maximum losses seem likely to occur, the crystallisation often taking weeks or even months.

In the case of neohesperidin dihydrochalcone preparation, it does not seem likely that the chalcone was incompletely formed. The experiments undertaken in which the effect of changes in alkali concentration and the effect of the duration of refluxation were examined do not support the case that incomplete chalcone formation is responsible for low yields. As was the case in the preparation of naringin dihydrochalcone the problem appeared to be that the evaporation procedure was overlong and that there is difficulty in obtaining neohesperidin dihydrochalcone from solution., The efficiency of formation of neohesperidin chalcone from phloroacetophenone-4'-β-neohesperidoside (84) and isovanillin was checked by reaction these two compounds by heating them in equimolar amounts on a steam bath for one hour, cooling and acidifying and then extracting unreacted isovanillin into diethyl-ether. This method showed that 56 % of the isovanillin had not reacted.

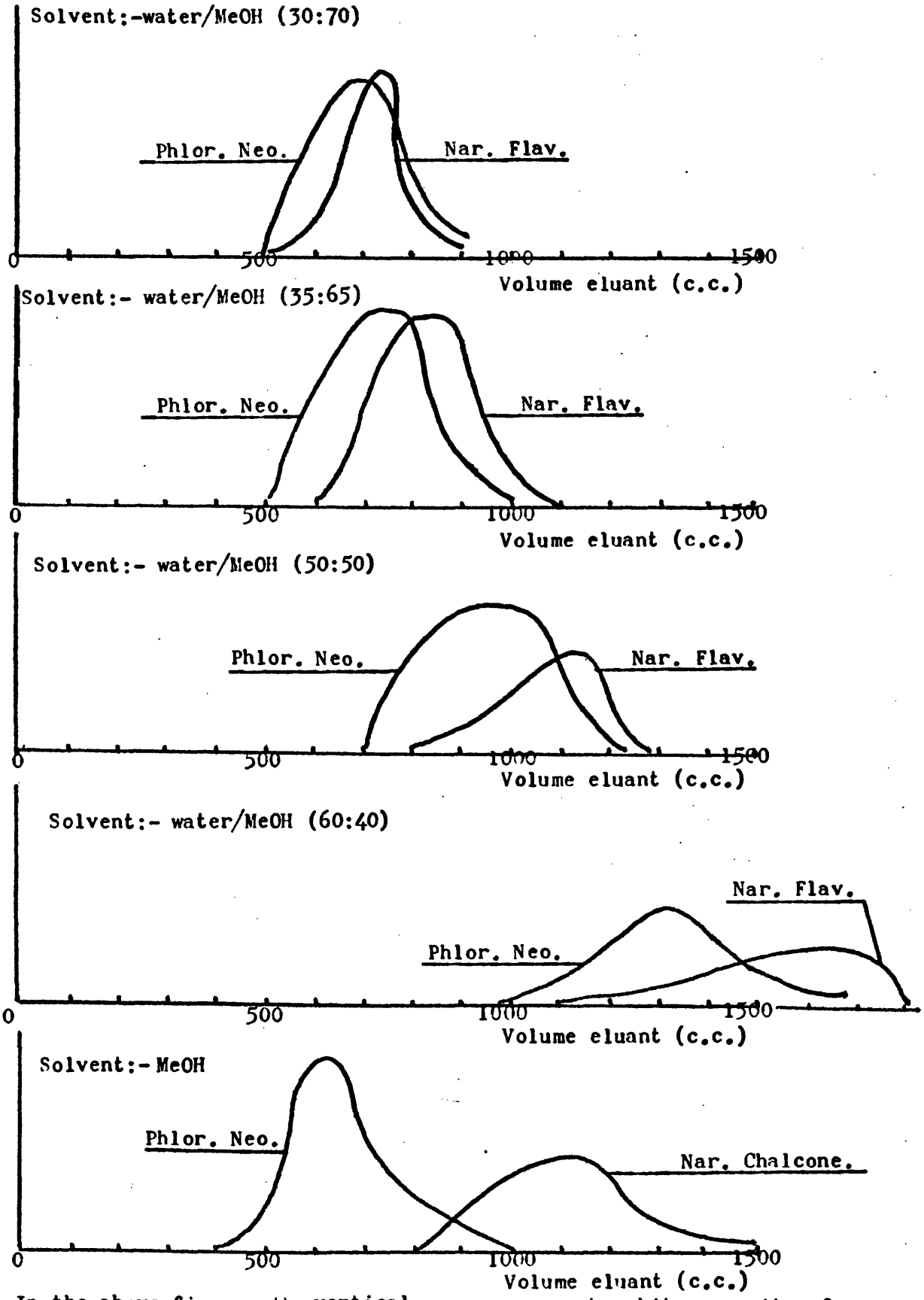
In all this work it was frequently necessary to use thin-layer chromatographic methods. At first, the eluants n-butanol/glacial acetic acid/water (4:1:5) and n-butanol/pyridine/water (10:3:3) were used with silica-gel-coated TLC plates but these systems gave poor separations between naringin chalcone and naringin flavanone. Also, naringin flavanone and phloroacetophenone-4'-β-neohesperidoside (84) have the same Rf value when these systems are used, although these two compounds can be distinguished by the fact that naringin flavanone is fluorescent whereas the phloroacetophenone compound is not. Better separations

were achieved with an eluant of methanol/water (60:40), and glass plates coated with polyamide. Horowitz also obtained better separations by using nitromethane/methanol (3:2). Typical Rf values for all these systems are shown in Table [11]

Before attempting to separate phloroacetophenone-4'-β neohesperidoside (84) and neohesperidin by column chromatography some trials were carried out with the phloracetophenone compound (84) and naringin flavanone (see page 139). The eluant methanol /water was investigated in proportions of methanol to water of 70:30, 65:35, 50:50 and 40:60 using a column filled with polyamide powder. Separations of the two above-mentioned compounds were not satisfactory. A reasonably satisfactory separation of the phloroacetophenone compound (84) and naringin chalcone was obtained, however, when methanol was used to elute these two compounds on a polyamide column, it was this latter system which was used, therefore, to separate neohesperidin chalcone from the phloroacetophenone compound (84). (see Figure 3)

A possible separation method was to carry out the reaction between the phloroacetophenone compound (84) and isovanillin, to hydrogenate the mixture and then to separate the phloroacetophenone compound (84), isovanillin and neohesperidin dihydrochalcone by column chromatography. To this end, a trial was conducted using naringin dihydrochalcone and the phloroacetophenone compound (84). A satisfactory separation was not obtained when a water/methanol (40:60) eluant was used with a polyamide column.

FIGURE 3 . COLUMN CHROMATOGRAPHY OF PHLOROACETOPHENONE-4'-β-NEOHESPERIDOSIDE, WITH EITHER NARINGIN FLAVANONE OR NARINGIN CHALCONE.



In the above figures the vertical axes represent arbitrary units of quantity. Abbreviations:- Phlor. Neo. is Phloroacetophenone-4'-β-neohesperidoside and Nar. Flav. is Naringin Flavanone.

For the purpose of making determinations of the concentration of phloroacetophenone-4'-β-neohesperidoside (84) in a mixture of this compound and naringin flavanone, an analytical method was developed (see pages[143] and[144]). The principle of this method is to stand a solution of the mixture in alkaline solution whereupon the naringin flavanone is converted to the chalcone and its absorbance at its wavelength maximum of 430 nm is determined. The phloroacetophenone compound absorbs at 295 nm and a correction is required when analysing such a mixture, because the chalcone contributes to the ketone peak at 295 nm. Even after correction, the experimentally determined concentrations of the ketone were widely divergent from the actual concentration in made-up mixtures. The experimentally determined concentrations of naringin chalcone were usually ca. 1 mg/100 cc greater than the actual concentrations which were between 10.50 and 16.70 mg/100 cc in made-up mixtures.

The Formulation and Sensory Evaluation of Chekwate and Quosh Orange Drinks containing Dihydrochalcone Sweeteners

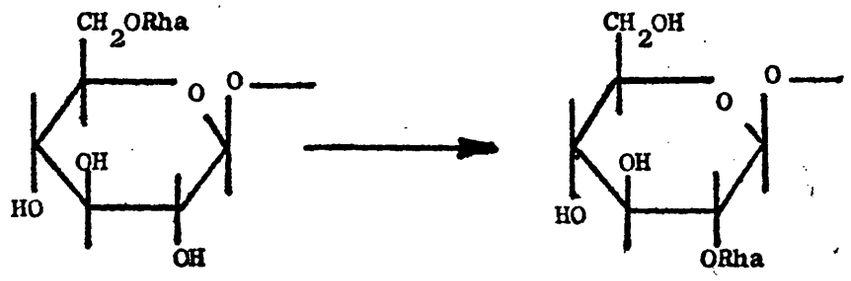
Chekwate Orange Drink was made up, sweetened with either neohesperidin dihydrochalcone, naringin dihydrochalcone and, for comparison saccharin & 'Aspartame'. Of eleven tasters, the majority preferred the standard product, unsweetened product being preferred to the dihydrochalcone-sweetened products. The Chekwate sweetened with neohesperidin dihydrochalcone was not liked because it exhibited delayed sweetness, gasp effect and its sweetness was

of a lingering, cloying nature. The dihydrochalcone sweetness remained in the mouth and had the effect of carrying over when the unsweetened Chekwate was tasted, so that the latter product also tasted sweet. Some tasters also detected an anaesthetic effect on the roof of the mouth and at the back of the throat. Naringin dihydrochalcone had similar taste characteristics to the neohesperidin dihydrochalcone, although this sweetener was not sweet enough even in a saturated solution. Tasters preferred the 'Aspartame' sweetener because its taste was purer than the dihydrochalcone although some tasters did detect a 'chemical' off-note with the former material. The same remarks which have applied to the Chekwate drinks also apply to the Quosh drinks although the sucrose in the latter product did tend to mask the dihydrochalcone. The sweetness intensities of the above mentioned sweeteners in both Chekwate and Quosh Orange drinks are listed in Tables[15] &[16] .

The Attempted Conversion of Hesperidin to Neohesperidin by Contacting the Hesperidin with Macerated Grapefruit

A whole grapefruit was macerated with hesperidin, allowed to stand and then treated with alkali to extract the flavonoids. Thin-layer chromatography did not reveal any difference between the extract obtained from the above sample and an extract obtained from grapefruit which had not been treated with hesperidin. The purpose of carrying out this experiment was to attempt to convert hesperidin, which contains a rutinose moiety, to neohesperidin, which possesses

a neohesperidose disaccharide, by a possible enzymic action. Does grapefruit contain an enzyme which is capable of effecting a migration of the rhamnose portion of rutinose from the 6-position, thus resulting in the formation of neohesperidose? This particular experiment gave a negative result, but the topic of the enzymic conversion of hesperidin to neohesperidin is probably worth further exploration.



Rutinose Neohesperidose

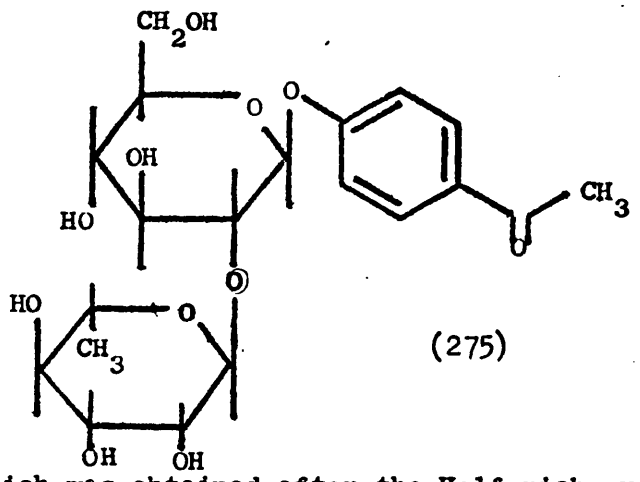
The Attempted Syntheses of Dihydrochalcone Disaccharides from Simple Starting Materials

Naringin and Neohesperidin are the naturally-occurring compounds from which sweet dihydrochalcones can be prepared, but their availability depends on collecting and processing the peel of grapefruits or Seville oranges, respectively. The total synthesis of dihydrochalcone disaccharides from the simple starting materials phloroglucinol, isovanillin, glucose and rhamnose would be a more desirable route for the commercial production of dihydrochalcone sweetener, providing satisfactory yields can be realised and providing that the overall process is economically viable. With these thoughts in mind, several attempts were made to synthesise several dihydrochalcone disaccharides from simple starting materials.

The first disaccharide which was investigated was neohesperidose. 1,3,4,6-tetraacetyl-α-D-glucopyranose (152) was prepared as a pale yellow syrup using the method of Helferich[92]. D (+) glucose was added to acetic anhydride and α-acetobromoglucose was formed in situ by adding phosphorus tribromide. Shaking the resulting syrup with aqueous sodium acetate resulted in the migration of an acetyl group from the 2-position of the glucose to the 1-position resulting in a tetra-acetyl glucose compound in which the hydroxyl group in the 2-position was available for substitution. Because the Helferich method had yielded a syrup following the in-situ preparation of α-acetobromoglucose a second preparation was attempted in which solid α- acetobromoglucose was stirred in aqueous sodium acetate solution. The solid was not totally dissolved and even after stirring overnight the only solid which was obtained was shown by its IR spectrum to be the starting material, α-acetobromoglucose.

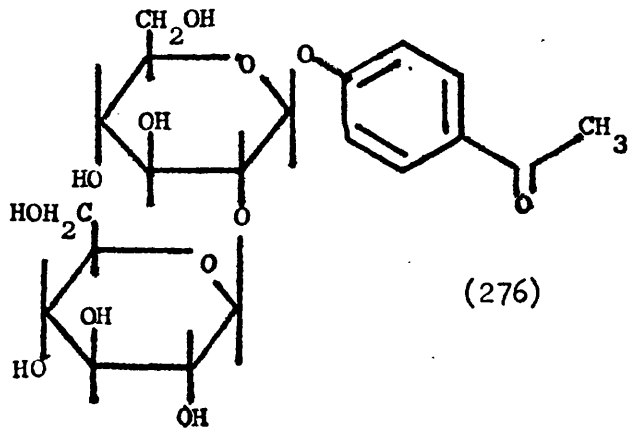
The syrup which was obtained by the Helferich method was reacted with 2,3,4-tri-O-acetyl-α-L-rhamnosyl bromide (153) in an attempted synthesis of hexaacetyl-α-neohesperidosyl bromide (156). The rhamnose compound (153) was prepared by the method of Fisher[169], that is by acetylating rhamnose and by bubbling hydrogen bromide gas into the mixture. A portion of the syrup obtained at the conclusion of the preparation of 1,3,4,6-tetraacetyl-α-D-glucopyranose (152) was added to 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide (153), dissolved in acetonitrile and mercuric cyanide and mercuric bromide added. The heptaacetyl-α-neohesperidose (154) failed to crystallise so the syrup was

dissolved in acetic anhydride and hydrogen bromide gas was bubbled into the mixture in order to prepare hexaacetyl-α-neohesperidosyl bromide (156). A brown syrup resulted which failed to crystallise after its storage in a refrigerator for one week. This syrup was added to 4-hydroxyacetophenone, dissolved in chloroform and silver carbonate catalyst added. Another syrup resulted, the compound 4-hydroxyacetophenone-4-β-neohesperidoside (275) was not isolated neither was its acetyl derivative.



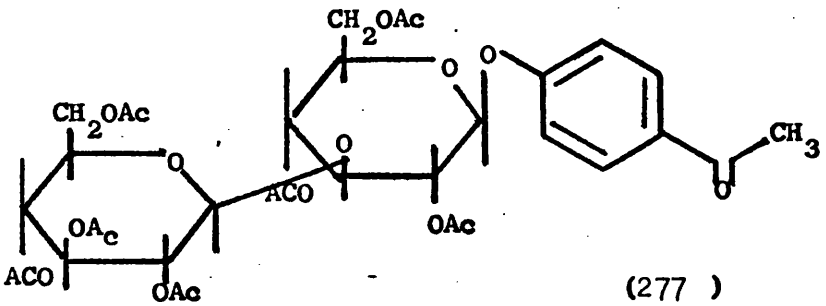
(275)

The syrup which was obtained after the Helferich synthesis of 1,3,4,6-tetra-O-acetyl-α-D-glucopyranose (152) was also reacted with α-acetobromoglucose in order to form octaacetyl-α-sophorose. Again, hydrogen gas was bubbled into the solution in order to form α-sophorosyl bromide, which was also obtained as a syrup. 4-hydroxyacetophenone was added to this syrup, together with silver carbonate catalyst to yield a brown syrup from which no crystals or solid material of 4-hydroxyacetophenone-4-β-sophoroside (276) were obtained neither was its acetyl derivative.



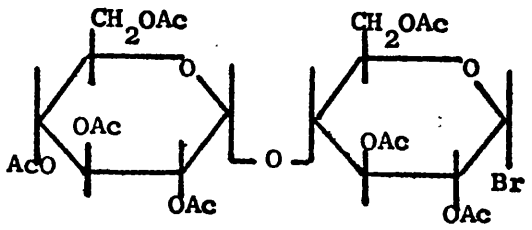
(276)

In another experiment the subject of interest was the synthesis of a dihydrochalcone in which the disaccharide moiety was laminaribiose or 3-O-β-D-glucopyranosyl-β-D-glucopyranose. To this end, the first compound required in such a synthesis was diacetoneglucose (1,2-4,6-di-isopropylideneglucopyranose) (168) which was prepared by shaking D(+) glucose in acidified acetone in the presence of anhydrous copper sulphate. The diacetoneglucose was added to benzene together with α-acetobromoglucose and silver carbonate to form the substituted laminaribiose compound (169). Subsequent treatment was carried out to remove isopropylidene groups, to form octa-acetyllaminaribiose (170), and finally, α-acetobromolaminaribiose (171). No solid was obtained at any of these stages. Reaction of the α-acetobromolaminaribiose with 4-hydroxyacetopenone failed to yield 4-hydroxyacetophenone-4-β-heptaacetyllaminaribioside (277).



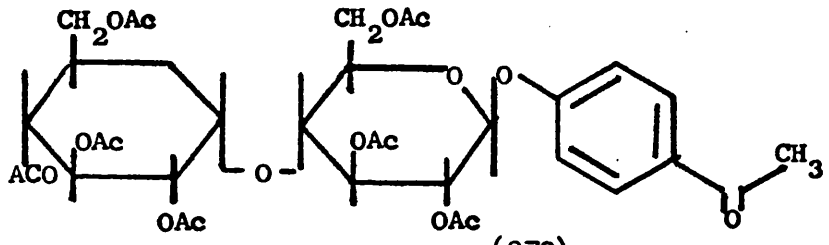
(277)

A dihydrochalcone maltoside is another compound of interest. Maltose (4-O-α-D-glucopyranosyl-D-glucopyranose) was acetylated and converted to α-acetobromomaltose (278) as a syrup.



(278)

The synthesis of 4-hydroxyacetophenone-4-O-β-D-heptaacetylmaltoside (279) was attempted by reacting brown syrup described above with 4-hydroxyacetophenone. The resulting syrup was subject to a chromatographic purification treatment to yield four fractions. A white solid was obtained, but the Rf value of this material seems rather higher (0.70) than would be expected for the compound (279).



(279)

Suggestions for Further Work

Enough work has been done on the dihydrochalcone glucosides based on the 4'-hydroxydihydrochalcone aglycone to demonstrate that these compounds are not sweet. Further work is worthwhile on the dihydrochalcone glucosides based on the 2',4'-dihydroxydihydrochalcone aglycone.

The total synthesis of dihydrochalcone disaccharides from simple starting materials as a commercially viable operation seems unlikely. Chemical glycosylation reactions yield the required disaccharides or glycosides in relatively low yields ($<$30%) although some improvement might be possible by the use of enzymes. The naringin or neohesperidin molecules

are just^{too} complex to be thinking of large-scale production of a sweetener which ought to be a cheap alternative to sucrose.

Synthesis of glucose/rhamnose and glucose/glucose disaccharides and incorporation into a dihydrochalcone aglycone would still be a useful academic exercise. These compounds and similar compounds in which selective hydroxyl groups have been blocked by methyl groups, would probably throw light on the mechanisms by which sweet substances exert their effect on the human taste buds.

EXPERIMENTAL

A) The Preparation of Dihydrochalcone Glycosides from naturally-occurring Naringin.

Naringin chalcone (8).

Naringin (2g,3.4mM) and 40cc. of 25% potassium hydroxide in water were allowed to stand overnight at room temperature, then cooled to 0°C, acidified to pH6 with concentrated hydrochlorie acid, filtered and dried in a dessicator m.p.164-170°C (naringin flavanone m.p. lit.[4] 165-8°C). IR spectrum identical to that for naringin flavanone ν_{max} cm.$^{-1}$, 3500 (OH) 1640 (C O), 1450,1295, 1265, 1200, 1180, 1130, 985, 880, 830, 810. Rf 0.61 (naringin flavanone 0.59). Plates-0.25mm. silica gel. Solvent-4:1:5 n-butanol/glacial acetic acid/water, top organic layer used. Detection-UV fluorescence.The preparation was repeated but the acidification step was omitted. Naringin (5g,8.6mM) was dissolved in 100cc. of 25% potassium hydroxide in water. The solution was allowed to stand at room temperature for two hours, then cooled, washed with water, air-dried and finally dried in a desiccator. Yield 6.9g(100%, therefore hydrated.) Rf 0.68, (naringin flavanone Rf 0.68) and 0.71, yellow (naringin chalcone). TLC method as described above.

Naringin dihydrochalcone (80).

Naringin (20g,34.5mM) was dissolved in 400cc. water and 50g. potassium hydroxide were added. The solution was allowed to stand at room temperature for two hours, then cooled to 0°C. A yellow precipitate of naringin chalcone resulted which was filtered, washed with water and partially dried in a desiccator.

The damp naringin chalcone was dissolved in 400cc. ethanol and 2g. of 10% palladium on carbon catalyst added. The mixture was hydrogenated at room temperature and atmospheric pressure. (Actual hydrogen uptake 780 cc. in 3 hours, theoretical uptake 772cc.). The ethanolic solution was evaporated nearly to dryness on a rotary evaporator and then transferred to an evaporating basin and heated on a steam-bath for 30 minutes. Some black slime separated which was thought at first to be colloidal carbon but its colour was brown-black, not pure black in colour. The evaporation continued overnight at room temperature in a fume-hood to yield a mass of pale yellow crystals. The solid was collected and recrystalised four times from water to yield silvery-white, fibrous crystals. These were dried at $<80^{o}C$ in an oven. Yield 9.7g. (48%). m.p. 162-4°C (lit.[48] 168-9°C). ν_{max} $cm.^{-1}$, 1630 (>C=O) 1515, 1435, 1365, 1300, 1235, 1200, 1180, 1130, 1065, 980, 820. $\lambda_{max}(\varepsilon)$nm., 283 (28,400). NMR (DMSO) ppm.,7.1 doublet [2] J=8.5Hz (C_2-H, C_6-H); 6.7 doublet [2] J=8.5Hz (C_3-H, C_5-H); 6.07 singlet [2] (C_3,-H, C_5,-H); 5.5 to 2.5 complex multiplet [24] (rhamnoglucosyl, phenolic and methylene protons). [Found:C,54.4; H,6.4; $C_{27}H_{34}O_{14}$ requires: C,55.7 H,5.8; $C_{27}H_{34}O_{14}.H_2O$ requires: C,54.0; H,6.0.]

Some data regarding five preparations of naringin DHC are shown in Table

Table[9] Some data concerning Naringin DHC preparation.

No	Yield %	m.p. °C	actual hydrogen uptake(cc)	theoretical hydrogen uptake (cc)	duration of hydrogenation
1	12	164-6	34.5	38.6	40m
2	33.5	162-8	105	193	75 m
3	48	162-4	780	772	3h
4	52	163-8	1100	773	2h
5	12	161-5	8115	5806	10h

Isolation and Purification of Naringin Flavanone ex Grapefruit Base.

The pale yellow precipitate of naringin flavanone was sieved from twelve barrels of2:1 comminuted grapefruit base prior to paste-milling. This material was dissolved in two litres of hot water at a temperature of 80°C, the solution was filtered and allowed to cool overnight. The crystals were collected by Buchner filtration and dried at 30°C for a period of one week.Yield-400g. Rf 0.209 (Rf of'Sunkist' naringin 0.212). Plates - precoated polyamide. Solvent - 40% methanol/water. Detection - fluorescence in UV light.

Phloroacetophenone-4'-β-neohesperidoside (84).

Naringin flavanone (125g,0.216M) was added to a solution of 200g. potassium hydroxide in one litre of distilled water. The mixture was stirred at room temperature for one hour and 15 minutes and then refluxed for another 1.25 hours. The reaction mixture was cooled to 10°C and acidified with 6N hydrochloric acid until a pH of 6.0 was reached, when a flocculent cream precipitate was thrown down. The mixture was allowed to stand overnight in the refrigerator and the solid was collected by Buchner filtration and recrystallised from methanol. Yield 77g.(51%). In successive preparations yields of 47, 58, 64 and 51% were obtained. m.p. 155-160°C. (lit.[4] 164-6°C) ν_{max} $cm.^{-1}$,1620 (>C=O), 1435, 1360, 1280, 1170, 1125, 1065, 975, 810. $\lambda_{max}(\varepsilon)$nm., in neutral ethanol 215 (13,600), 228 (15,100), 285 (16,800); in 0.01M KOH/EtOH 245(sh) (14,300), 295 (13,400), 373 (5200). NMR (DMSO) ppm., 6.15 singlet [2] (C_3-H,C_5-H); 5.5 to 3.0 complex multiplet [20] (glycosidic and phenolic protons); 2.65 singlet [3] (Ar.CO.CH_3); 1.25 doublet [3] J=6Hz ($-CH_3$ of rhamnose). [Found: C,47.9; H,6.7; $C_{20}H_{28}O_{13}$ requires: C,48.7; H,6.1; $C_{20}H_{28}O_{13}.2H_2O$. requires: C,47.0;H,6.3.] A mass spectrum was not possible because sample decomposition took place on the hot probe. Rf 0.63 (Rf naringin chalcone 0.70, Rf naringin flavanone 0.64). TLC plates -0.1mm. silica gel. Solvent - n-butanol/pyridine/water 10:3:3.

Detection - naringin flavanone, UV-fluorescent; naringin chalcone, yellow; phloroacetophenone-4'-β-neohesperidoside, brown spot on standing for four days or iodine vapour reveals a brown spot almost immediately. Second alternative TLC, Rf 0.53 (Rf naringin chalcone 0.64, Rf naringin flavanone 0.55).TLC plates - 0.25mm. silica gel. Solvent - n-butanol/glacial acetic acid/water 4:1:5, top organic layer used. Detection - as described above or a benzidene spray reagent gives a red/brown colouration with flavonoids. Third alternative TLC, Rf 0.35 (Rf naringin flavanone 0.40). TLC plates - precoated polyamide. Solvent 3:2 nitromethane/methanol. Detection - as described above. After the first preparation of phloroacetophenone-4'-β-neohesperidoside, the spot produced by this compound during TLC on silica gel was confused with that of naringin flavanone until it was realised that the spot due to the phloroacetophenone compound was not fluorescent whereas that for the flavanone was. It was at first thought that the iso-vanillin was recombining with the phloroacetophenone compound until this was disproved by observation of the course of the alkaline hydrolysis of naringin by the withdrawal of samples and the examination of their UV spectra. The chalcone absorption at 440nm. diminished as the heating proceeded until after two hours no further diminution took place and the reaction was judged complete. A rapid test which may be carried out on a solid which is either phloroacetophenone-4'-β-neohesperidoside or naringin flavanone is to record its UV spectrum and to note the shift of the major peak on making the solution alkaline. Thus, the phloroacetophenone compound shifts from 285nm. to 295nm. on making alkaline whereas naringin flavanone shifts from 285nm. to 430nm. (see Spectra 7 & 8.)

The Preparation of Neohesperidin Dihydrochalcone from Phloroacetophenone-4'-β-neohesperidoside(81).

Phloroacetophenone-4'-β-neohesperidoside (5g,10.5mM) was dissolved in 10cc. methanol and isovanillin (1.6g,10.5mM) was added. 100cc. of 50% potassium hydroxide in water was poured into the mixture and the resulting solution was heated on a steam-bath for one hour.There was no immediate precipitation when the solution was cooled but an orange precipitate was slowly thrown down over a period of one week in a refrigerator. This was recrystallised from water to yield 3g. (46.7%) of a yellow solid. λ_{max} nm., 277, 313, 372 (sh). The yellow solid(2g.) was dissolved in 20cc. methanol and 0.5g. of 10% palladium/carbon catalyst was added. This mixture was hydrogenated until the required amount of hydrogen was utilised (75cc.), then it was filtered to remove the catalyst and rotary-evaporated to yield 0.7g (35%) of a sweet,white solid. m.p.150-4°C (lit[48,4] 153-155°C,152-154°C). ν_{max} $cm.^{-1}$, 3400 (-OH), 1620 (>C=O), 1590, 1505, 1430, 1265, 1120,1060, 1020, 970, 800, 755. Rf 0.24. TLC plate - precoated polyamide. Solvent - 3:2 nitromethane/methanol. Detection - benzidene spray.

Preparation of Neohesperidin Chalcone. A) The Effect of Alkali Concentration on the Yield of Chalcone.

Reaction mixtures were prepared containing accurately weighed quantities of phloroacetophenone-4'-β-neohesperidoside, isovanillin and potassium hydroxide. 50cc. water was then added to each mixture and the resulting solutions were refluxed for ten minutes in each case. After this treatment the solutions were cooled and their absorptions at 450nm. were read.(see Table 10).As the alkali concentration increased the absorption at 450nm. increased so that the maximum chalcone absorption was obtained when ca. 50% alkali was used.

Table 10. The Effect of Alkali Concentration on the Yield of Neohesperidin Chalcone.

Weight phloroacetophenone-4'-β neohesperidoside.(g)	Weight isovanillin (g)	Weight potassium hydroxide (g)	Absorbance at450nm
0.1004	0.1000	5	0.443
0.1009	0.1000	10	0.416
0.1002	0.1000	15	0.510
0.1010	0.1090	20	0.630
0.1005	0.1012	25	0.730

Preparation of Neohesperidin Chalcone.B) The Effect of Duration of Refluxation on the Yield of Chalcone.

0.1002g. of phloroacetophenone-4'-β-neohesperidoside and 0.1010g. isovanillin were added to 50cc. of 25% potassium hydroxide in water. The mixture was refluxed for one hour after which time the mixture was cooled, made up to 100cc. in a standard flask and the absorbance read at 450nm. i.e. 0.486. Compare the absorbance for a ten-minute refluxation, that is, between 0.416 and 0.51 (see Table 10).The conclusion is that a one-hour refluxation does not result in a higher yield of chalcone than a ten-minute one.

Preparation of Neohesperidin Chalcone. C) The Efficiency of Chalcone Formation

Phloroacetophenone-4'-β-neohesperidoside (5g,10.5mM) and Isovanillin (1.6g.,10.5mM) were dissolved in 50% potassium hydroxide in water (100cc.) and the solution was heated on a steam-bath for one hour. The mixture was then cooledand acidified to pH 6 whilst cooling. The solution was extracted with diethyl-ether (6 x 50cc.) and the ether extracts were evaporated to dryness. Weight of isovanillin recovered 0.89g. Therefore, the approximate % of isovanillin recovered and not reacted is 56%.

The Preparation of Neohesperidin Dihydrochalcone directly from Naringin.

Naringin (200g,0.34M) and isovanillin (106g,0.70M) were dissolved in two litres of 20% potassium hydroxide in water and the mixture was refluxed for four hours and thirty minutes. The mixture was then cooled overnight and 6M hydrochlorie acid was added until pH7 was reached. A brown oil of crude isovanillin precipitated and this was separated off. Potassium hydroxide (200g,0.34M) was added to the remaining solution so that its potassium hydroxide concentration was 10% w/v, the total volume of the solution now being ca.3 litres. 10g. of 5% palladium/carbon catalyst was added and the mixture was hydrogenated at room temperature and atmospheric pressure over a period of 120 hours until the uptake was 8.5 litres of hydrogen (theoretical uptake 7.7litres). The mixture was filtered to remove the catalyst, acidified to pH7 with 6M hydrochloric acid and the crystallised potassium chloride was separated, washed with methanol and the washings added to the main bulk of the liquor. The liquor was diluted to 300cc. with acetone and stored in a refrigerator. After about six months crystals were observed in the acetone solution. These were collected and dried in a desiccator. Yield of crude product 5.4g. (3%). m.p. 148-152°C.(lit[48,4] 153-155, 152-154°C).

The Thin-layer Chromatographic Separation of Naringin Flavanone and Chalcone, Neohesperidin Flavanone and Phloroacetophenone-4'-β-neohesperidoside.

The disadvantage of using the eluants n-butanol/glacial acetic acid/water (4:1:5) and n-butanol/pyridine/water (10:3:3) with silica gel coated TLC plates was that the separation between naringin chalcone and naringin flavanone was not great. Also, naringin flavanone and phloroacetophenone-4'-β-neohesperidoside have the same Rf in these solvents, although they can be distinguished by the fact that naringin flavanone is fluorescent whereas the phloroacetophenone compound is not.

In the present work, several methanol/water mixtures of varying composition were tried using polyamide coated on glass plates. The optimum separation was attained using 60:40 methanol/water. Horowitz also obtained better separations using polyamide plates and nitromethane/methanol eluant (3:2). Typical Rf values for all these TLC systems are shown in Table

Table[11] .Some Rf Values obtained by TLC for several Flavonoids.

	Silica Gel		Polyamide	
	n-BuOH/HOAc/H_2O 4:1:5	n-BuOH/py/H_2O 10:3:3	MeOH/H_2O 6:4	$MeNO_2$/MeOH 3:2
naringin flavanone.	0.55	0.64	0.58	0.57
naringin chalcone.	0.64	0.70	0.11	-
neohesperidin flavanone.	-	-	0.47	0.69
neohesperidin chalcone.	-	-	-	-
phloroacetophenone-4'-β-neohesperidoside.	0.53	0.63	0.65	0.48

The Column Chromatographic Separation of Phloroacetophenone-4'-β-neohesperidoside, Naringin Flavanone and Naringin Chalcone.

Before subjecting phloroacetophenone-4'-β-neohesperidoside/isovanillin reaction products to column chromatography in order to isolate pure neohesperidin, the chromatographic behaviour of the title compounds was investigated. The eluant methanol/water was investigated in proportions of methanol to water of 70:30, 65:35, 50:50 and 40:60 using a 4cm.✖45cm. glass column filled with polyamide (Woelm) powder for column chromatography. None of these eluants successfully separated mixtures of naringin flavanone from the phloroacetophenone compound which were both applied to the column in 0.5g. amounts, dissolved in the minimum quantity of methanol. 100cc. fractions of eluant were collected and diluted samples were subjected to UV and visible spectrophotometric analysis, absorption readings being obtained at wavelengths of 285nm. (the maxima for phloroacetophenone-4'-β-neohesperidoside and naringin flavanone), 370nm. (the maximum for naringin flavanone) and 430nm. (the absorption maximum for naringin chalcone).

The results are shown in Figure [3] . The separation of phloroacetophenone-4'-β-neohesperidoside and naringin chalcone was best achieved by eluting with methanol (see Figure).The attempted separation of the title compounds on polyamide by elution with methanol containing 0.25% sodium hydroxide was not possible. All three compounds were rapidly eluted as a single band (after 400 to 600cc. of eluant). It had been hoped that elution in an alkaline medium might have prevented any reversion of chalcone to flavanone, thus aiding separation.

The Column Chromatographic Separation of the Products of the Reaction of Phloroacetophenone-4'-β-neohesperidoside and Isovanillin.

Phloroacetophenone-4'-β-neohesperidoside (10g, 21mM) and isovanillin (10g,65.8mM) were added to 200cc. of 10% potassium hydroxide in water and the solution was allowed to stand at room temperature for 48 hours. The solution was then cooled to between 0°C and 10°C, neutralised to pH7 with concentrated hydrochloric acid and extracted with three 200cc. portions of diethyl ether to remove unreacted isovanillin.Then, 25cc. aliquots were applied to a 4cm.×50cm. column of polyamide powder and eluted with methanol. Unreacted phloroacetophenone-4'-β-neohesperidoside was eluted in the 700cc. to 800cc. fraction together with some neohesperidin flavanone. The chalcone was eluted as a broad band (1000cc. to 1600cc. fraction) and these latter fractions were evaporated by rotary evaporation under vacuum. Combination of the concentrates from six separations finally yielded a yellow powder of neohesperidin chalcone. Yield 0.1g. (0.78%). m.p. 188-194°C (lit.[48] 201-2°C). ν_{max} $cm.^{-1}$, 1630, 1570, 1510, 1440, 1360, 1270, 1200, 1170, 1130, 1070, 1020, 980, 910, 880, 810, 760, 735. These wavelength maxima agree very well with the values obtained by Kamiya (read from a spectrum, numerical figures not given) 1610, 1570, 1500, 1430, 1390, 1300, 1200, 1150, 1070, 920, 880, 850, 820, 770, 740,. λ_{max} nm. 375. NMR (DMSO) ppm., 7.6 to 6.9 complex multiplet [3] (aromatic protons of 'B' ring);

6.2 singlet [2] (C_3-H, C_5-H); 5.5 to 3.0 complex multiplet [13] (glycosidic protons); 3.9 singlet [3] (-OCH_3); 3.45 singlet [9] (-OH, removed by deuteration); 1.2 multiplet [3] (-CH_3 of rhamnose).

The Attempted Column Chromatographic Separation of a Mixture of Naringin Dihydrochalcone and Phloroacetophenone-4'-β- neohesperidoside.

0.1g. of naringin dihydrochalcone was applied to a 4cm.×45cm. glass column filled with polyamide (Woelm) and eluted with 40:60 water/methanol. The absorbance of the eluate at 285nm. was measured spectrophotometrically. The dihydrochalcone was eluted in the 600cc. to 1000cc. fraction. On a subsequent chromatographic run 0.1g. of phloroacetophenone-4'-β-neohesperidoside was also eluted in the 600cc. to 1000cc. fraction.

The Determination of the Solubilities in Water of Phloroacetophenone-4'-β-neohesperidoside and Naringin Flavanone.

Extinction coefficients for phloroacetophenone-4'-β-neohesperidoside and naringin flavanone were calculated by use of the formula:

$$\varepsilon = \frac{\text{absorbance} \times MW \times 100}{C}$$

where ε is the extinction coefficient, MW is the molecular weight and C is the concentration (mg./100cc.) of solute in water. For the phloroacetophenone compound ε =18,620, 19,030 for two successive determinations. ε_{mean} =18,825 ± 205 at 285nm. For naringin flavanone ε =18,180, 17,570 for two successive determinations. ε_{mean}=17,875 ± 305 at 285nm.

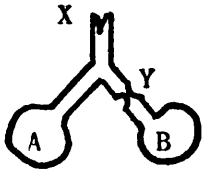
Test-tubes, each containing 5cc. distilled water were placed in a thermostatically-controlled water-bath at 65°C (actual temperature-63.5°C). Either solid was added to each until no more would dissolve, when the tubes and their contents were left for two hours to reach equilibrium. A pre-heated pipette was used to withdraw 0.5cc. into 50cc. standard volumetric flasks, after which the flasks were diluted to the mark with distilled water and mixed. After further dilution the absorbance at 285nm.

was read on a Unicam SP 800 recording spectrophotometer. Solubilities were calculated by use of the above-mentioned formula and by substituting the appropriate values for molecular weight and absorbance. The method was repeated in order to obtain solubilities at 21°C. The results are shown in Table[12] .

Table [12] Solubilities in Water of Phloroacetophenone-4'-β-neohesperidoside and Naringin Flavanone at 21°C and 63.5°C.

	Solubility at 21°C $g.l^{-1}$	Solubility at 63.5°C $g.l^{-1}$
Phloroacetophenone-4'-β-neohesperidoside.	0.90	7.8
Naringin flavanone.	12.0	91.0

The solubility of naringin flavanone at 63.5°C in water was checked using a bulb of a type shown in the diagram below:



The method used was as follows:

1) Approximately 0.1g. of solid and 2g. of liquid was placed in bulb A. The bulbs were then sealed at X.

2) The whole vessel was heated at 63.5°C for two hours.

3) Some supernate from bulb A was decanted into bulb B whilst holding the vessel in the thermostatically controlled water-bath. The bulb was then sealed at Y , allowed to cool to room temperature and then weighed.

4) The nipple at Y was removed and the liquid was evaporated in an oven at 50°C to dryness. The bulb plus the solid and the nipple were weighed.

5) The solid was washed from the bulb with acetone, the dried bulb was then weighed together with the nipple.

Two successive determinations gave solubilities of naringin flavanone in water at 63.5°C of 70.6$g.l^{-1}$ and 80.2$g.l^{-1}$. Mean solubility 75.4 ± 5.2$g.l^{-1}$.

The Determination of the Concentration of Phloroacetophenone-4'-β-neohesperidoside in a Mixture of this Compound and Naringin Flavanone.

If a mixture of the two title compounds is diluted with 0.1M potassium hydroxide in ethanol, their absorptions in UV and visible light may be determined spectrophotometrically. The flavanone is converted to the chalcone in this medium and absorbs at 430nm. (ε =19,290), the phloroacetophenone-4'-β-neohesperidoside absorbs at 295nm. (ε=9389).

$$\text{Since} \quad \varepsilon = \frac{\text{absorbance} \times MW \times 100}{C}$$

where is the extinction coefficient, MW is the molecular weight and C is the concentration (mg./100cc.).

$$\text{Concentration of phloroacetophenone-4'-}\beta\text{-neohesperidoside.} = \frac{\text{absorbance} \times 476 \times 100}{9389}$$

$$\text{Concentration of naringin flavanone} = \frac{\text{absorbance} \times 580 \times 100}{19,290}$$

The absorbances for phloroacetophenone-4'-β-neohesperidoside were in need of correction because of the contribution at 295nm. of a secondary peak due to the chalcone spectrum. The ratio of chalcone absorbance at 430nm. to the absorbance at 295nm. was found to be 4.18, 4.05, 4.18, Mean 4.14.

Therefore the corrected phloroacetophenone-4'-β-neohesperidoside absorption is as follows:

$$A_{corr.} = A_{obs.} - \frac{A_{430nm.}}{414}$$

where $A_{corr.}$ is the corrected absorbance, A_{obs} is the observed absorbance and $A_{430nm.}$ is the chalcone absorbance at 430nm. The results are shown in Table 13.

Table 13. A Comparison of the Actual Concentrations of Phloroacetophenone-4'-β-neohesperidoside and Naringin Flavanone with the Experimentally determined Values.

CONCENTRATION			
Actual	Actual	Exptl.	Exptl.
Phlor. neo. mg./100cc.	Nar. flav. mg./100cc.	Phlor. neo. mg./100cc.	Nar. flav. mg./100cc.
6.12	14.24	9.1	15.2
2.66	10.50	4.3	11.0
6.24	16.70	9.1	17.4

Table 14. Absorption Values from which the Concentrations shown in Table 13 were calculated.

Dilution in 0.1M KOH in EtOH	Absorbance at 430nm.	Absorbance at 295nm.	Corrected absorbance at 295nm.
5 times	1.01	0.60	0.36
"	0.73	0.35	0.17
"	1.16	0.64	0.36

B) The Formulation and Sensory Evaluation of Chekwate and Quosh Orange Drinks containing Dihydrochalcone Sweeteners.

Chekwate Orange Drink was made up and 26fl.oz. samples of the drink were sweetened with 0.2g./26fl.oz. neohesperidin dihydrochalcone or 2.0g./26fl.oz. naringin dihydrochalcone and, for comparison, 0.64g/26fl.oz. saccharin and 1g./26fl.oz. 'Aspartame' sweetener (L-aspartyl-L-phenyl-alanine methyl ester). Of the eleven tasters, the majority preferred the standard product, unsweetened product being preferred to the dihydrochalcone-sweetened products. The Chekwate sweetened with neohesperidin dihydrochalcone was generally not liked for its delayed sweetness, its gasp effect and its lingering, cloying nature. The dihydrochalcone sweetness remained in the mouth and had the effect of carrying over when the unsweetened Chekwate was tasted so that the latter product also tasted sweet. Some tasters also noticed an anaesthetic effect on the roof of the mouth and at the back of the throat. The naringin dihydrochalcone was not

sweet enough even at a concentration of 2g/26fl.oz. in the Chekwate product. Its effects were substantially identical with those of neohesperidin dihydrochalcone but they were less marked. The 'Aspartame' -sweetened Chekwate Orange Drink was generally liked for its purity of taste and virtual absence of taste side effects. Some tasters did detect a 'chemical' off-note, however.

Quosh Orange Drink was made up and 26fl.oz. samples of the drink were sweetened with 0.2g./26 fl.oz. neohesperidin dihydrochalcone, 0.2g/26fl.oz. of the calcium salt of neohesperidin dihydrochalcone or 1.0g./26 fl.oz. of the sodium salt of naringin dihydrochalcone. The standard Quosh contained 0.384g./26fl.oz. of saccharin whilst the saccharin was excluded from the dihydrochalcone-sweetened drinks. Seven tasters tasted these drinks and the majority preferred the saccharin-sweetened standard product. The dihydrochalcone-sweetened drinks were not liked for their gasp effect and their lingering, cloying sweetness. The neohesperidin DHC-sweetened Quosh Orange Drink was superior to the Neohesperidin DHC-sweetened Chekwate Orange Drink owing to the masking effect, presumably, of the sucrose sweetness in the former product. Of the three dihydrochalcones tested, the calcium salt of the neohesperidin DHC was totally unacceptable owing to a strong off-flavour described as 'rubbery' or 'liquorice-like'. The sodium salt of naringin DHC was not sweet enough at 1g./26fl.oz. and the neohesperidin DHC did possess the undesirable gasp effect and lingering sweetness. Aspartame sweetener was also added to Quosh Orange Drink at a concentration of 1g./26fl.oz. from which saccharin had been omitted. At this concentration the drink was sweeter than the saccharin-sweetened standard product. The Aspartame-sweetened drinks were preferred over the dihydrochalcone-sweetened drinks for their purity of taste although two of the five tasters did detect a chemical off-note.

The Sweetness Intensities of Dihydrochalcone and Aspartame Sweeteners.

The sweetness intensities of several dihydrochalcones and Aspartame sweeteners were compared with the sweetness intensities of saccharin-sweetened Quosh and Chekwate Orange Drinks. In carrying out these comparisons the standard products were tasted first, followed by the drinks containing the sweeteners under test. The concentrations of sweeteners in the Quosh and Chekwate drinks and the equivalences of the sweetnesses of these sweeteners to saccharin at these concentrations are shown in Tables[15] and 16.

Table[15]. Sweetness Equivalences in Quosh Orange Drink.

Sweetener	Concentration of sweetener (g./26fl.oz.) in Drink equivalent in sweetness to Standard Quosh containing 0.38g./26fl.oz. saccharin.	Sweetness Equivalence
Neohesperidin DHC	0.1	3.8× saccharin
Naringin DHC	2.0	0.19× saccharin
Naringin DHC (sodium salt)	2.0	0.19× saccharin
Aspartame	0.5	0.76× saccharin

Table[16].Sweetness Equivalences in Chekwate Orange Drink.

Sweetener	Concentration of sweetener (g./26fl.oz.) in Drink equivalent in sweetness to Standard Chekwate containing 0.64g./26fl.oz. saccharin.	Sweetness Equivalence
Neohesperidin DHC	0.1	6.4× saccharin
Naringin DHC	3.0	0.21× saccharin
Naringin DHC (sodium salt)	3.0	0.21× saccharin
Aspartame	0.5	1.28× saccharin

C)The Attempted Conversion of Hesperidin to Neohesperidin by contacting the Hesperidin with Macerated Grapefruit.

One whole grapefruit was macerated in a Kenwood liquidiser and 0.5g. of hesperidin was mixed in. The mixture was allowed to stand overnight at room temperature then divided into two halves. One half was treated with 250cc. of 10% potassium hydroxide in water immediately. The second half was also treated in the same way after the mixture had been standing for three days. Each alkaline extract was acidified to pH7 and allowed to stand overnight in the refrigerator. Yellow precipitates had separated and portions of these were dissolved in methanol and subjected to TLC examination. Both the spots obtained for firstly, the mixtures described above and secondly, for a blank maceration and extraction which was carried out on a grapefruit to which no hesperidin had been added,were of the same Rf value i.e. 0.09. Hesperidin flavanone gave an Rf of 0.42. Hesperidin chalcone gave an Rf of 0.12. TLC plates - precoated polyamide. Solvent - 3:2 nitromethane/methanol. Detection - Benzidene Spray. i.e. Mix two parts of solution (2) with three parts of solution (1) where solution (1) is made up of 5g. benzidene or 6g. benzidene hydrochloride stirred with 14cc. concentrated hydrochloric acid diluted to 980cc. with water. Solution (2) is made up of 10g. sodium nitrite in 100cc. water. This solution is sprayed immediately after mixing. Therefore no difference was detected between the chromatograms of the samples with or without added hesperidin.

D) The Attempted Syntheses of Dihydrochalcone Disaccharides from Simple Starting Materials.

1,3,4,6-tetraacetyl-α-D-glucopyranose[152].

D(+)-glucose (132g,0.67M) was added gradually during 45 minutes whilst cooling to acetic anhydride (500cc,5.7M) to which 40 drops of 70% perchloric acid had been added. The solution was allowed to stand for one hour, then cooled to 15°C and stirred whilst phosphorus tribromide (86cc, 0.91M) was added dropwise,the temperature being kept at < 25°C. Water was added (46cc.) (temp. < 25°C) and the solution was allowed to stand at room temperature for 1.5 hours. Then, after cooling to 10°C, 100cc. of a solution of sodium acetate trihydrate in water (400g. in 500cc.) was added, whilst cooling and stirring and keeping the temperature below 45-50°C.The remaining 400cc. of the sodium acetate solution was then poured in. The pale yellow solution was extracted with 200cc. chloroform followed by three 100cc. extractions. The chloroform extracts were washed with water, aqueous sodium bisulphite and finally with water again. After drying with anhydrous calcium chloride the chloroform was evaporated off during rotary-evaporation to yield a pale yellow syrup which failed to crystallise. m.p. (lit.[92] 98-100°C). Yield ca. 250cc. of syrup.

2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide[153].

L(+)-rhamnose (25g,0.137M) and sodium acetate (10g,0.122M) were added to acetic anhydride (90g,0.88M) and the mixture was heated on a steam-bath for 15 minutes. Its colour darkened to light brown and the mixture solidified on cooling. The crystals were redissolved by warming and hydrogen bromide gas was bubbled into the mixture until a weight gain of 55g. was achieved. The mixture was allowed to stand overnight in a refrigerator and the hydrogen bromide, acetic acid and acetic anhydride were removed by rotary evaporation using a water-bath at 60°C. Di-iso-propyl ether was added and the mixture was allowed to stand overnight in a refrigerator to yield white crystals. Yield 18.8g. (54.0%). m.p. 67-70°C (lit[169] 71-72°C).

The Attempted Synthesis of Hexaacetyl-α-neohesperidosyl bromide (156).

1,3,4,6-tetraacetyl-α-D-glucopyranose (10cc. syrup, see page 148) was added to 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide (153) (5g, 14.2mM) in 100cc. acetonitrile together with mercuric cyanide, $Hg(CN)_2$ (1.8g, 7.12mM) and mercuric bromide, $HgBr_2$ (2.6g, 7.21mM). After 3 hours at room temperature the solvent was evaporated and the residue was treated with chloroform (200cc.) and filtered to remove the mercury salts. The filtrate was extracted with 1M potassium bromide in water (3×50) and water (3×100). The chloroform was evaporated to yield a brown syrup which failed to crystallise which was dissolved in methylene dichloride (10cc.) and glacial acetic acid (10cc) and acetic anhydride (5cc.) were added. Hydrogen bromide gas was bubbled into the solution until the gain in weight was 10g. and the mixture was allowed to stand for 20 minutes at room temperature. Then, the solution was diluted with chloroform (200cc.) and the whole was washed with water (2×100), saturated sodium bicarbonate solution in water (2×50) and water (3×100). The chloroform solution was dried with anhydrous calcium chloride, and evaporated to dryness at 30°C by rotary evaporation. A brown syrup resulted which failed to crystallise after leaving in a refrigerator for one week. Yield ca. 12cc. of syrup.

The Attempted Synthesis of 4-hydroxyacetophenone-4-β-neohesperidoside (175).

The brown syrup, described in the preceeding section, probably containing hexaacetyl-α-neohesperidosyl bromide, was added to 4-hydroxyacetophenone (2g, 14.7mM) and silver carbonate (4g, 14.5mM) in chloroform (30cc.). The mixture was stirred at room temperature overnight, then the silver oxide was filtered off and the filtrate evaporated under vacuum to yield a brown syrup. Dissolution in 30cc. chloroform and extraction with water (30cc.) resulted in the recovery of 67% of the 4-hydroxyacetophenone. After drying the chloroform layer with anhydrous calcium chloride and evaporating off the solvent under vacuum no crystalline material could be obtained. A syrup resulted.

The Attempted Synthesis of Heptaacetyl-α-sophorosyl bromide (165).

1,3,4,6-tetraacetyl-α-D-glucopyranose (10cc. syrup, see page 148) was added to α-acetobromoglucose (153) (5g,15.1mM) in 100cc. acetonitrile together with mercuric cyanide, $Hg(CN)_2$ (1.9g,7.52mM) and mercuric bromide, $HgBr_2$ (2.7g,7.48mM). After standing at room temperature for 3 hours the mixture was treated in exactly the same manner as was the case for the attempted hexaacetyl-α-neohesperidosyl bromide synthesis (see page 149). Finally, a dried chloroform solution was obtained which on evaporation yielded a dark brown syrup from which no crystalline material could be obtained following conventional cooling and trituration procedures. Yield of syrup ca. 20cc.

The Attempted Synthesis of 4-hydroxyacetophenone-4-β-sophoroside (276).

The syrup, described in the preceeding section, probably containing heptaacetyl-α-sophorosyl bromide, was added to 4-hydroxyacetophenone (2.1g, 15.4mM) and silver carbonate (4.5g, 16.3mM) in chloroform (30cc.). The mixture was treated in the same fashion as was the case for the attempted synthesis of 4-hydroxyacetophenone-4-β-neohesperidoside. The resulting chloroform solution was dried and evaporated to yield a dark brown syrup from which no crystals or solid material crystallised.

1,2-4,6-di-isopropylideneglucopyranose or diacetoneglucose (168).

D(+)-glucose (90g,0.5M) and anhydrous copper sulphate (200g, 1.25M) were added to acetone (2 litres) and concentrated sulphuric acid (10cc.) contained in a stoppered Winchester bottle. The mixture was shaken at room temperature for two days, filtered to remove the copper sulphate and the filtrate neutralised with ammonium hydroxide. The solution was again filtered and the filtrate evaporated under reduced pressure to yield 72.8g. (56%) of a white solid. m.p.106-8°C (lit. 110°C)[111] ν_{max} $cm.^{-1}$, 3450,1320,1290,1250,1220,1160,1120,1090,1060,1030,1005,940,930,880,840, 775.

3-O-β-D-glucopyranosyl-β-D-glucopyranose or laminaribiose (see page 42)

Diacetoneglucose (10g, 3.84M), together with silver carbonate(15g,5.43M) and calcium sulphate (30g, 0.22M) were added to benzene (80cc.) and shaken for 12 hours. Then, iodine (3g, 1.18M) and α-acetobromoglucose (15.7g,4.45M) were added to dry benzene (80cc.) and this solution was added to the mixture described above. The whole mixture was shaken at room temperature for 3 days, then filtered and extracted with water (4×100). The benzene layer was dried with anhydrous sodium sulphate and evaporated under vacuum to yield 25cc. of a pale yellow syrup which failed to yield any solid material following the usual cooling and trituration procedures. This syrup was dissolved in methanol (80cc.), therefore, and chromatography was carried out using a 4×50 cm. column of cellulose. Eluting solvent, n-butanol/pyridine/water 6:1:1 , The material obtained from the various fractions is summarised in Table[17] .

Table[17]. The Chromatographic Separation of the Products obtained in the Laminaribiose Preparation.

No.	Fraction cc.	Results
1	0-1800	no solid.
2	1800-1950	some gummy deposit.
3	1980-2370	0.91g. white solid, some gummy yellow solid also.
4	2400-2760	0.24g. white solid.
5	2790-3150	0.18g. white solid.

All the white solid which was obtained from fractions 1980-3150 by evaporating each fraction under vacuum using a water-bath at a temperature of $< 30^{\circ}C$, had the same appearance. TLC of these fractions 3,4 and 5 was carried out using silica gel TLC plates and n-butanol/acetic acid/water 4:1:5 as the developing solvent. Detection- 50% concentrated sulphuric acid/ethanol spray followed by heating in an oven at $100^{\circ}C$ for 10 minutes. Spots appeared as grey areas.Thus, fraction 3 yielded two spots of Rf values 0.19 and 0.38 (Rf for D(+)-glucose 0.38); fractions 4 and 5 each yielded a single spot of Rf 0.18. The Rf for diacetoneglucose was 0.90.

Thus, it is highly likely that this spot of Rf 0.18 is produced by the substituted laminaribiose compound 3-O-β-D-(2',3',4',6'-tetraacetyl-glucopyranosyl)-1,2-4,6-diisopropylideneglucopyranose (169).

The Attempted Synthesis of 4-hydroxyacetophenone-4-O-β-heptaacetyl-laminaribioside. (277).

The white solid which was obtained from the last two fractions, i.e. numbers 4 and 5, of 0.42g. weight was dissolved in0.1% sulphuric acid (100cc.) warmed on a steam-bath for three hours. The solution was evaporated to a syrup and acetic anhydride (50cc) was added. The mixture was heated on a steam-bath for five hours and evaporated to a pale yellow syrup. No crystals of octaacetyl-laminaribiose were obtained. The syrup was redissolved in acetic anhydride (25cc.) and hydrogen bromide gas (10g.) was bubbled into the solution. Subsequent evaporation yielded a brown syrup which was added to 4-hydroxyacetophenone (0.2g.). The title compound was not isolated.

α-acetobromomaltose (278).

Maltose monohydrate (99.5g, 0.28M) and sodium acetate (50g, 0.61M) were added to acetic anhydride (500g, 4.9M) and the solution was heated on a steam-bath for two hours. The mixture was then poured into two litres of ice and water and extracted with chloroform (3×300). The chloroform extract was washed with water (200cc.), 1%w/v sodium bicarbonate solution in water (200cc.) and finally with water (200cc.). After drying with anhydrous calcium chloride the chloroform was evaporated under reduced pressure to yield a syrup which on cooling became a glass. The glass was warmed with ethanol (500cc.) to dissolve, then cooled and allowed to stand overnight in the refrigerator but no crystallisation took place. Therefore, the ethanol was evaporated off and acetic anhydride(150cc.) was added. Gaseous hydrogen bromide (110g.) was bubbled into the mixture whilst cooling to 10°C in ice/water. After allowing it to stand for 60 hours the mixture was evaporated under reduced pressure and then stored overnight in the refrigerator. No crystallisation resulted. The black syrup was, therefore, subjected to a chromatographic purification procedure by eluting it on a column of silica gel (5×60cm.) using 5% acetone in diethyl ether as the eluting solvent. After evaporation under reduced

pressure of the eluted fractions a brown syrup was collected (50cc.) which failed to crystallise.

The Attempted Synthesis of 4-hydroxyacetophenone-4-O-β-D-heptaacetylmaltoside (279).

A portion of the brown syrup (30g.) described in the previous section was added to 4-hydroxyacetophenone (6.0g, 44.1mM) and dry calcium chloride (20g, 0.18M). The mixture was stirred for 3 days and then subjected to a chromatographic separation procedure by eluting it on a column of silica gel (5 60cm.) using diethyl ether, 10%v/v acetone/diethyl ether or acetone as the developing solvents. The material obtained from the various fractions is summarised in Table[18].

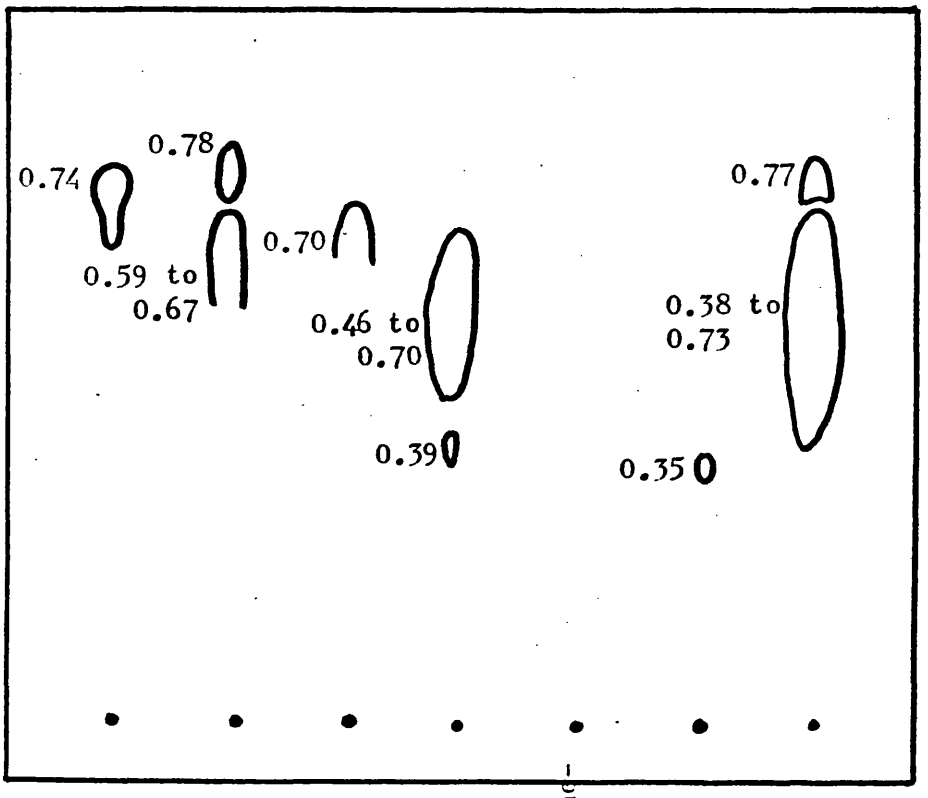
Table18 .The Chromatographic Separation of the Products obtained in the Preparation of 4-hydroxyacetophenone-4-O-β-D-heptaacetylmaltoside ( ).

No.	Fraction	Eluant	Results
1	0- 500	diethyl ether	dark brown syrup
2	500-2000	diethyl ether	pale yellow syrup white ppt.
3	2000-2500	10%acetone/ether	brown syrup
4	2500-4500	acetone	pale yellow syrup

A white solid was obtained from the second fraction only. TLC of these fractions and the starting materials was carried out using silica gel plates, n-butanol/acetic acid/water 4:1:5 as the eluant. Detection- 50% concentrated sulphuric acid/ethanol spray followed by heating in an oven at 100°C for 10 minutes. The TLC chromatogram is reproduced below:

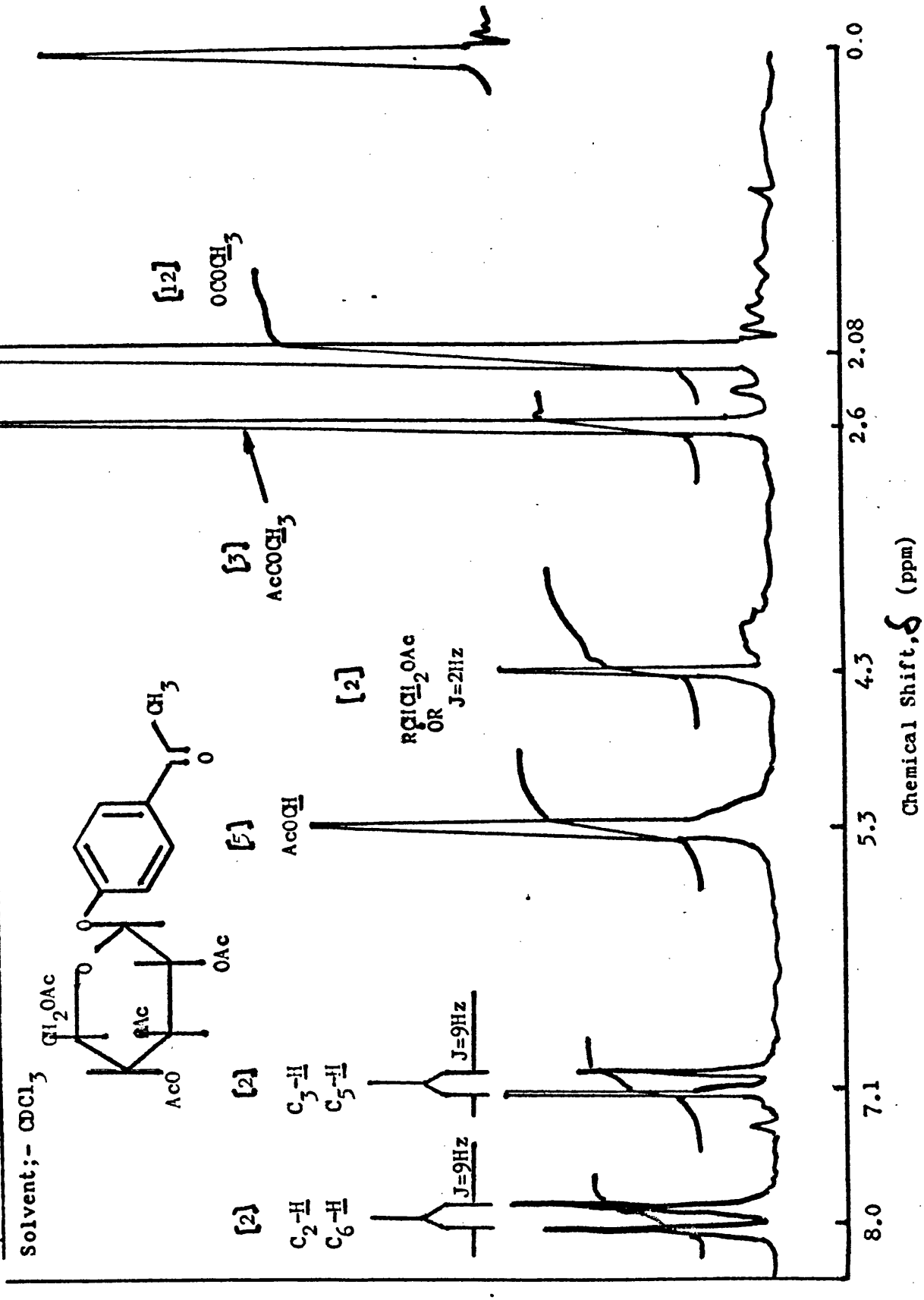
m.p. of white solid, indefinite between 64 and ca. 90°C. I.R. spectrum, ν_{max} $cm.^{-1}$, 3500 (OH), 1744 (OAc), 1244, 1154, 1130, 1050, 945, 904, 895.

DIAGRAM. The Chromatographic Separation of the Products obtained in the Preparation of 4-hydroxyacetophenone-4-O-β-D-heptaacetylmaltoside (279).

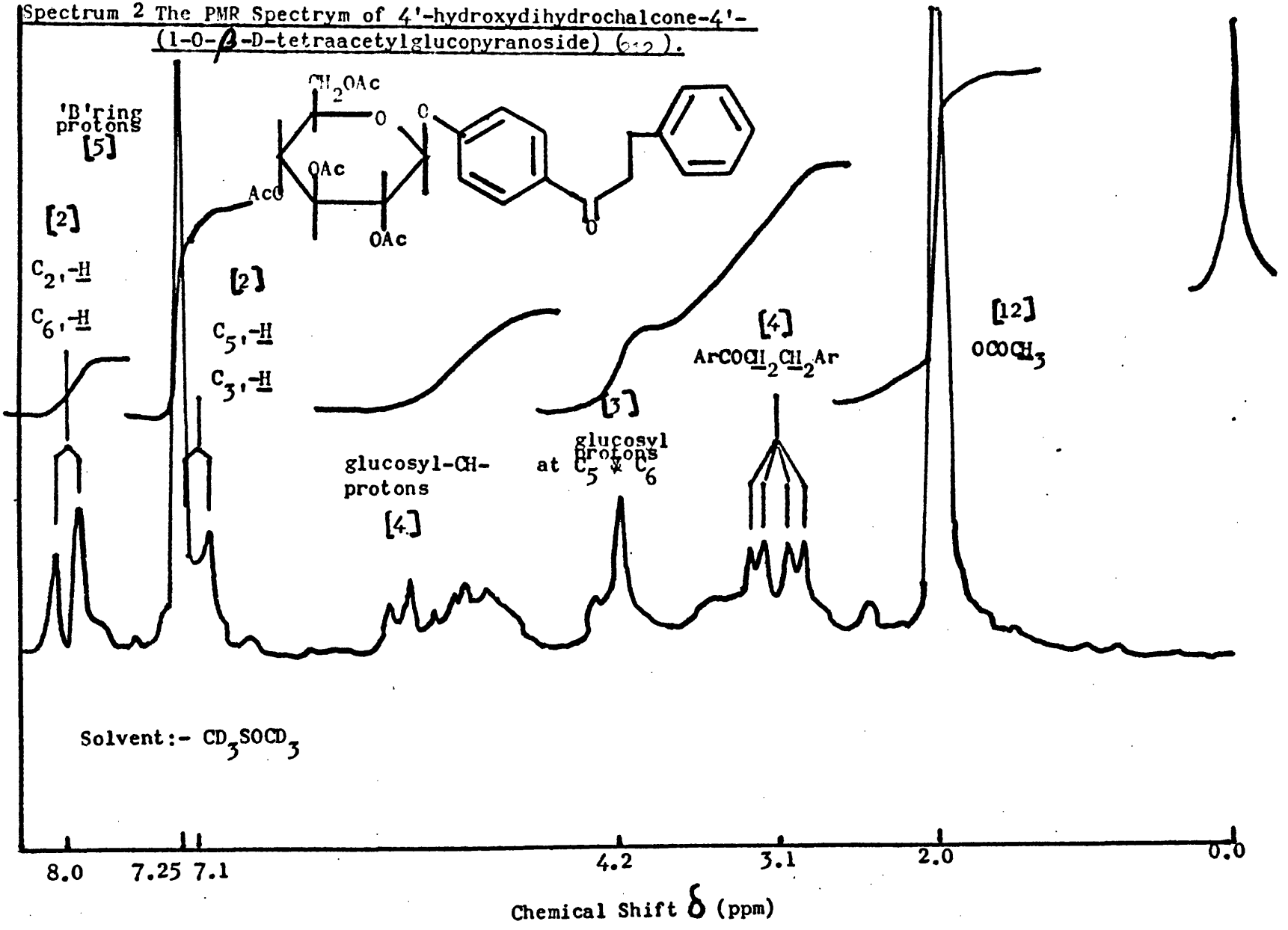


PART D

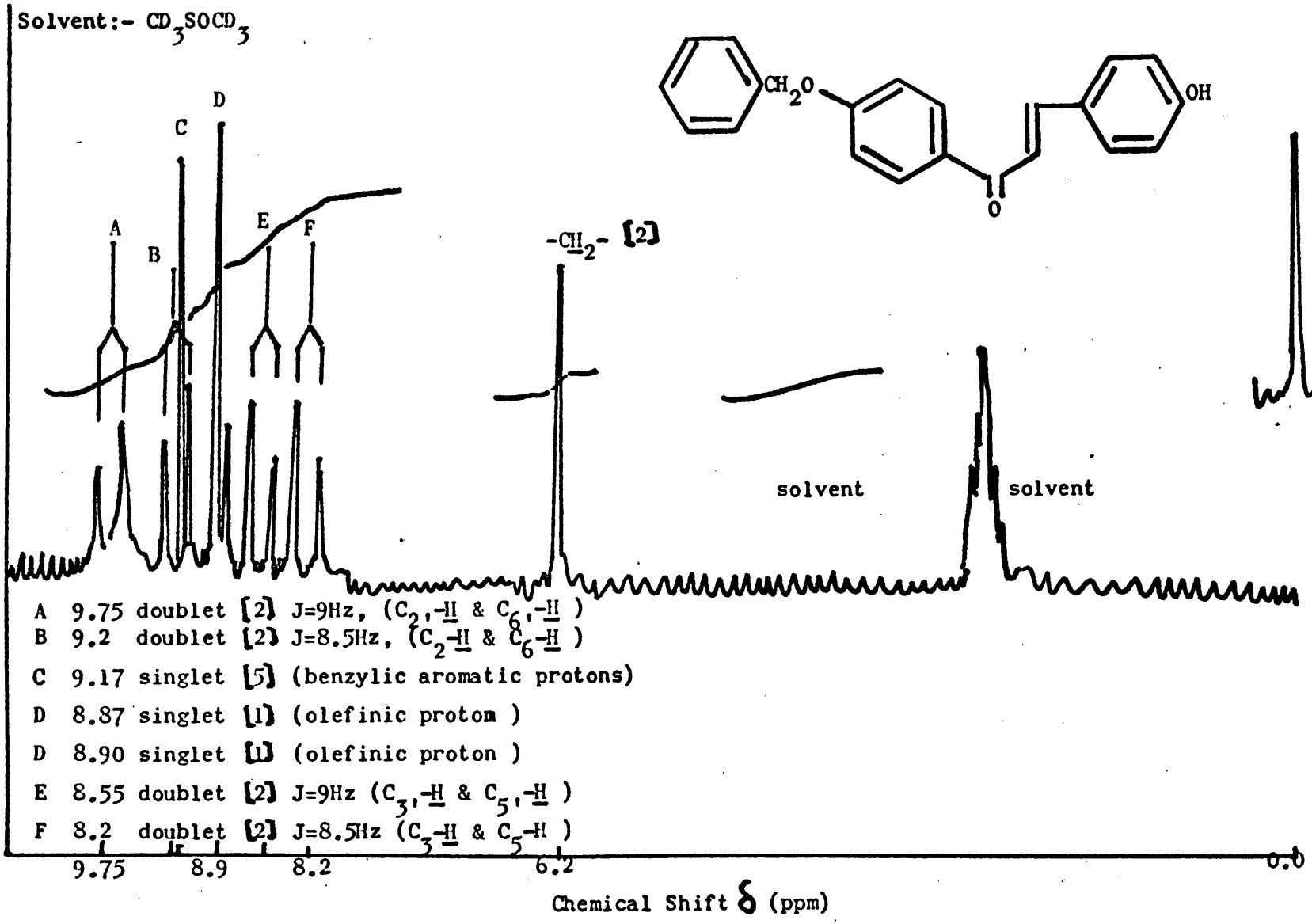
SPECTRA


Spectrum1 The PMR Spectrum of tetraacetylpiccin (253)
Solvent;- CDCl3
[12]
OCOCH3
[3]
AcCOCH3
[2]
RCH2CH2OAc
OR
J=2Hz
[1]
AcOCH
[2]
C3-H
C5-H
J=9Hz
[2]
C2-H
C6-H
J=9Hz
8.0
7.1
5.3
4.3
2.6
2.08
0.0
Chemical Shift, δ (ppm)

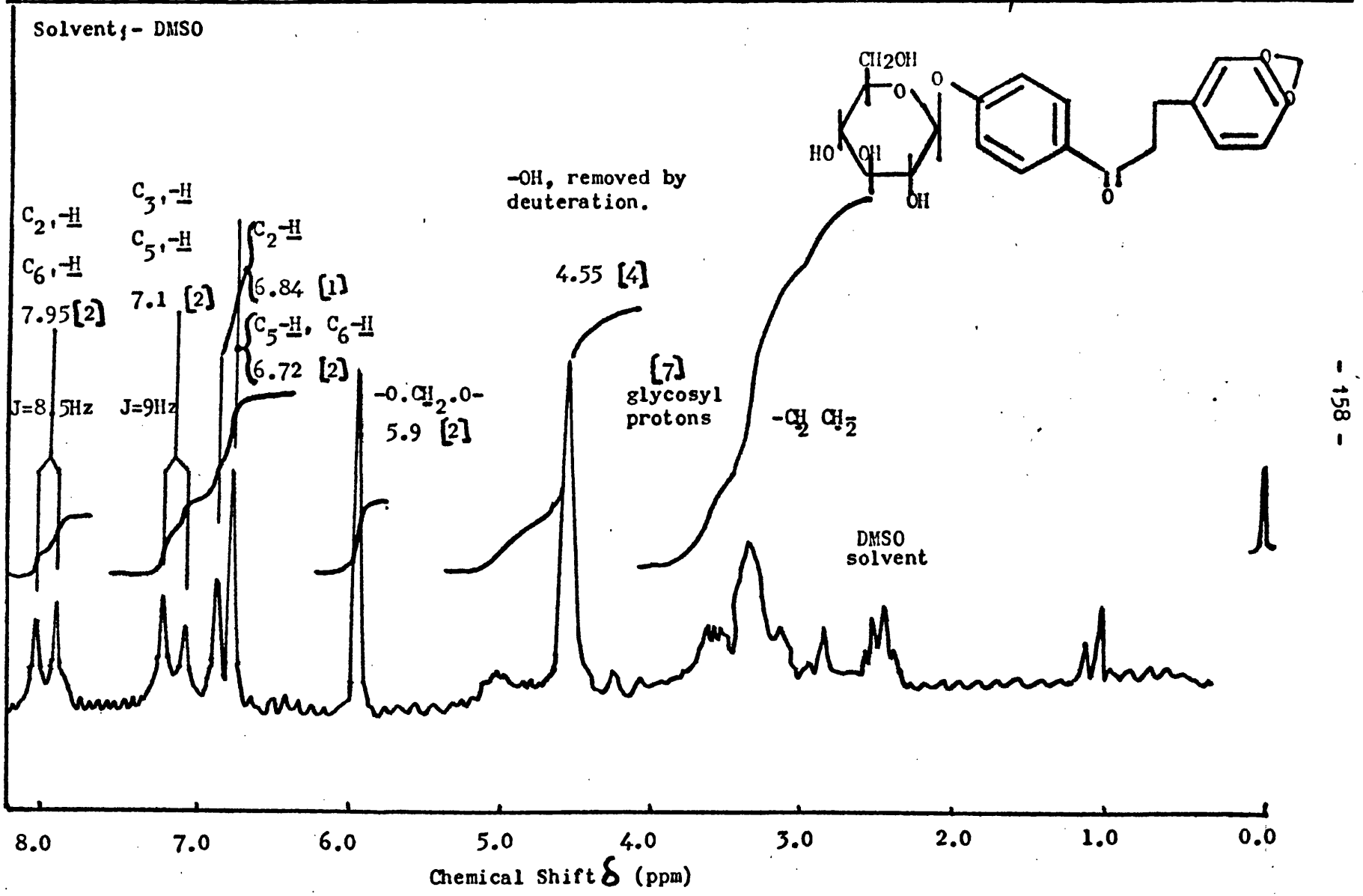
Spectrum 2 The PMR Spectrym of 4'-hydroxydihydrochalcone-4'-(1-O-β-D-tetraacetylglucopyranoside) (6:2).



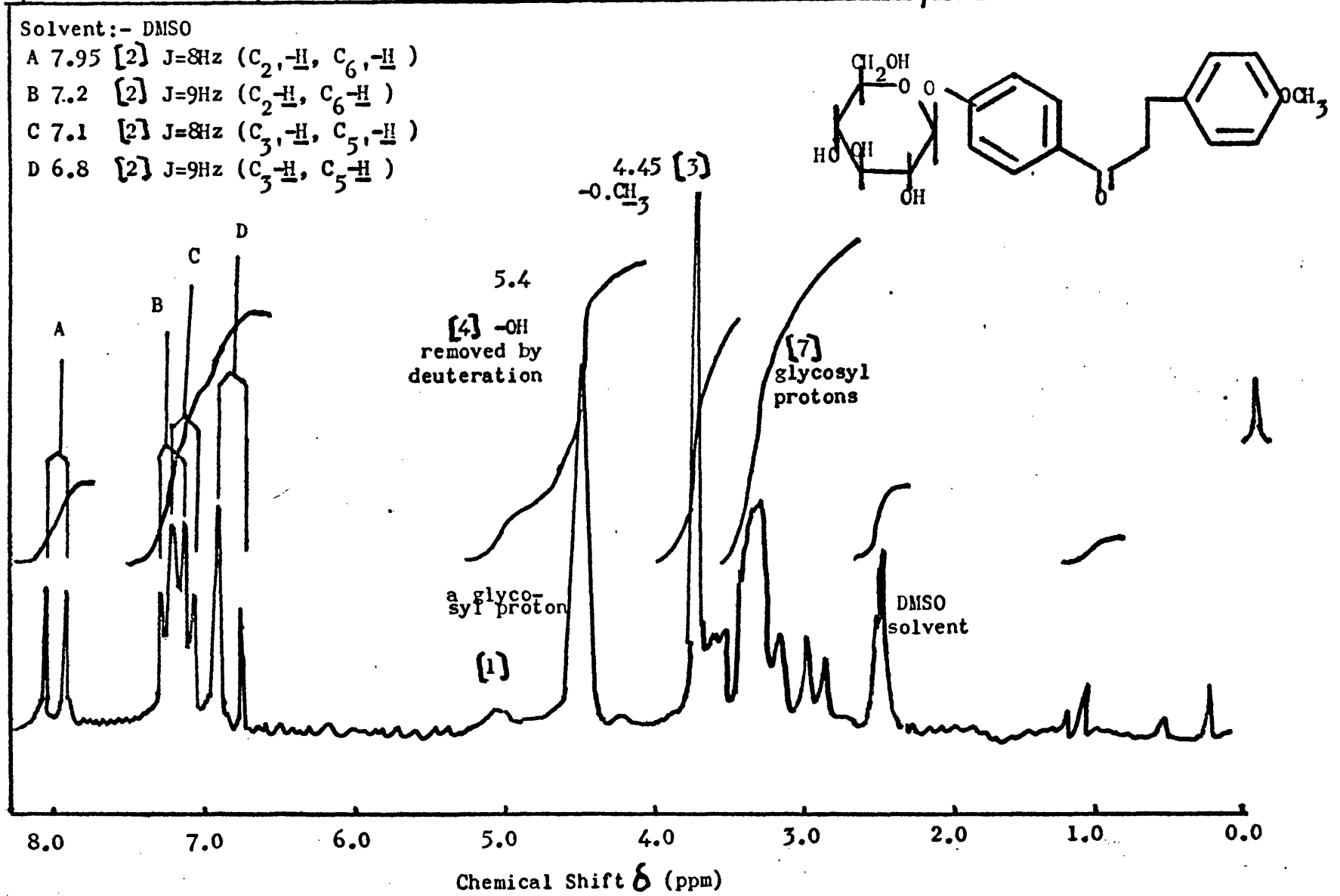
Spectrum 3 The PMR Spectrum of 4-hydroxy-4'-benzyloxychalcone (232)



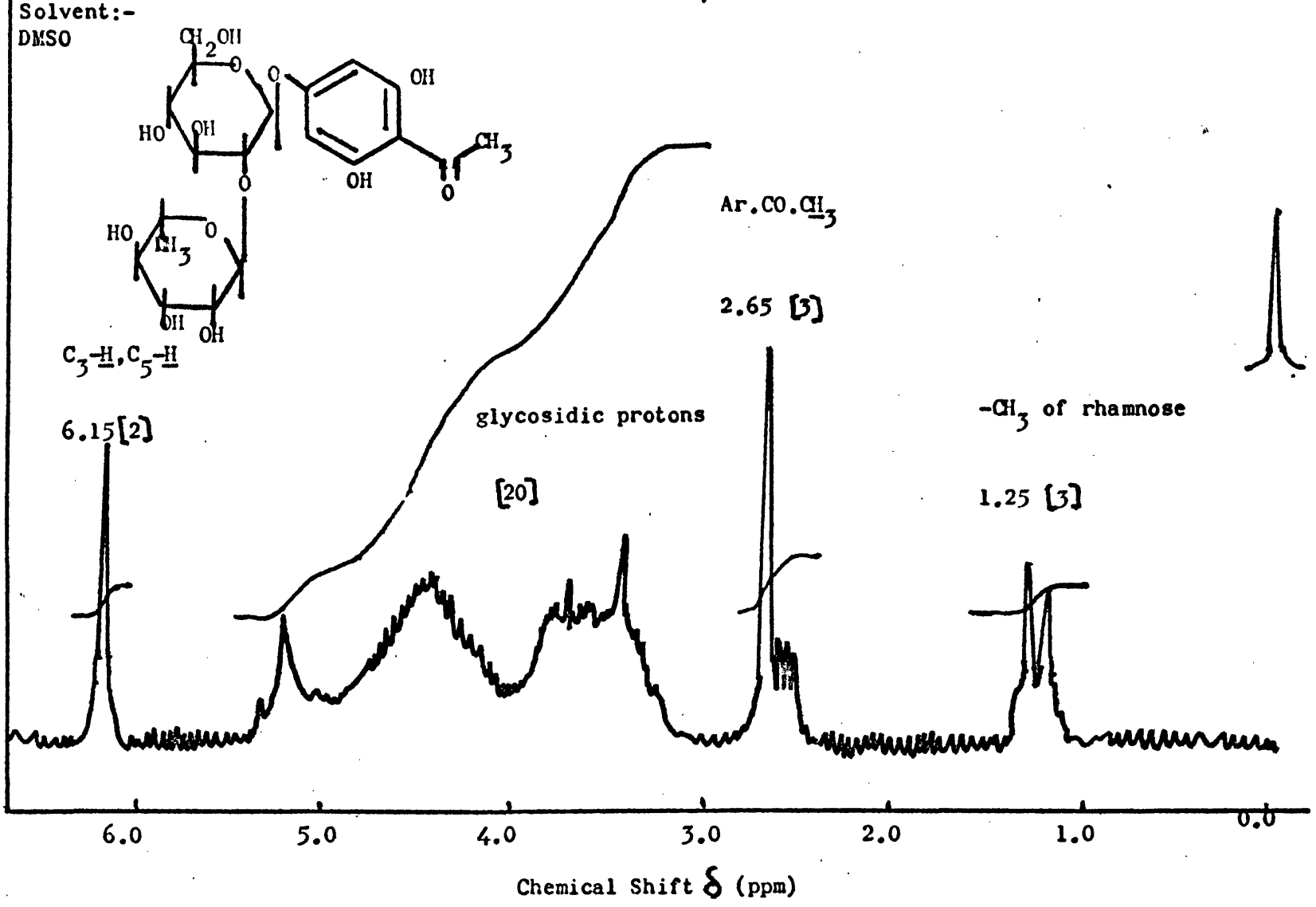
Spectrum4 The PMR Spectrum of 4'-hydroxy-3,4-methlenedioxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (226).



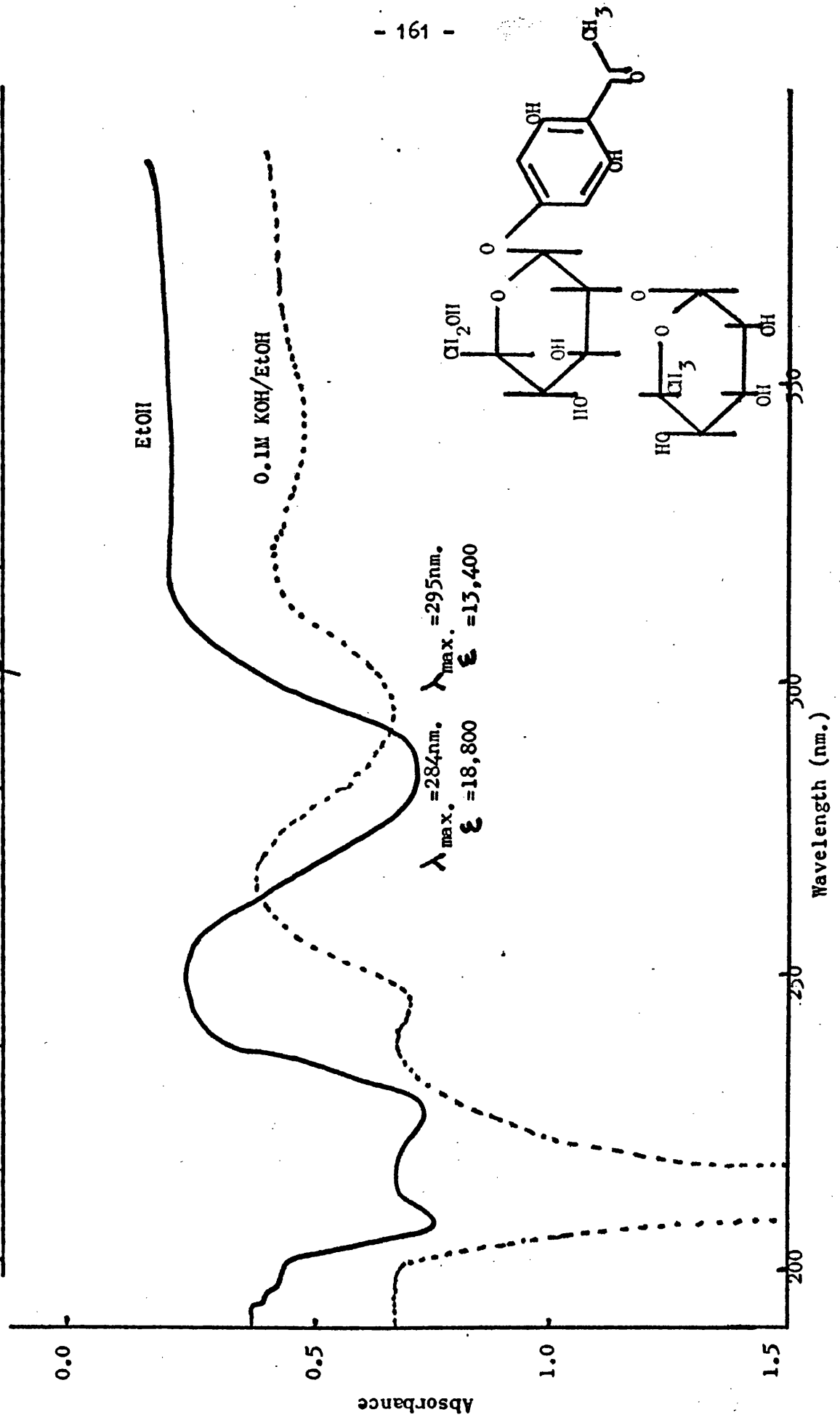
Spectrum 5 The PMR Spectrum of 4'-hydroxy-4-methoxydihydrochalcone-4'-(1-O-β-D-glucopyranoside) (260).



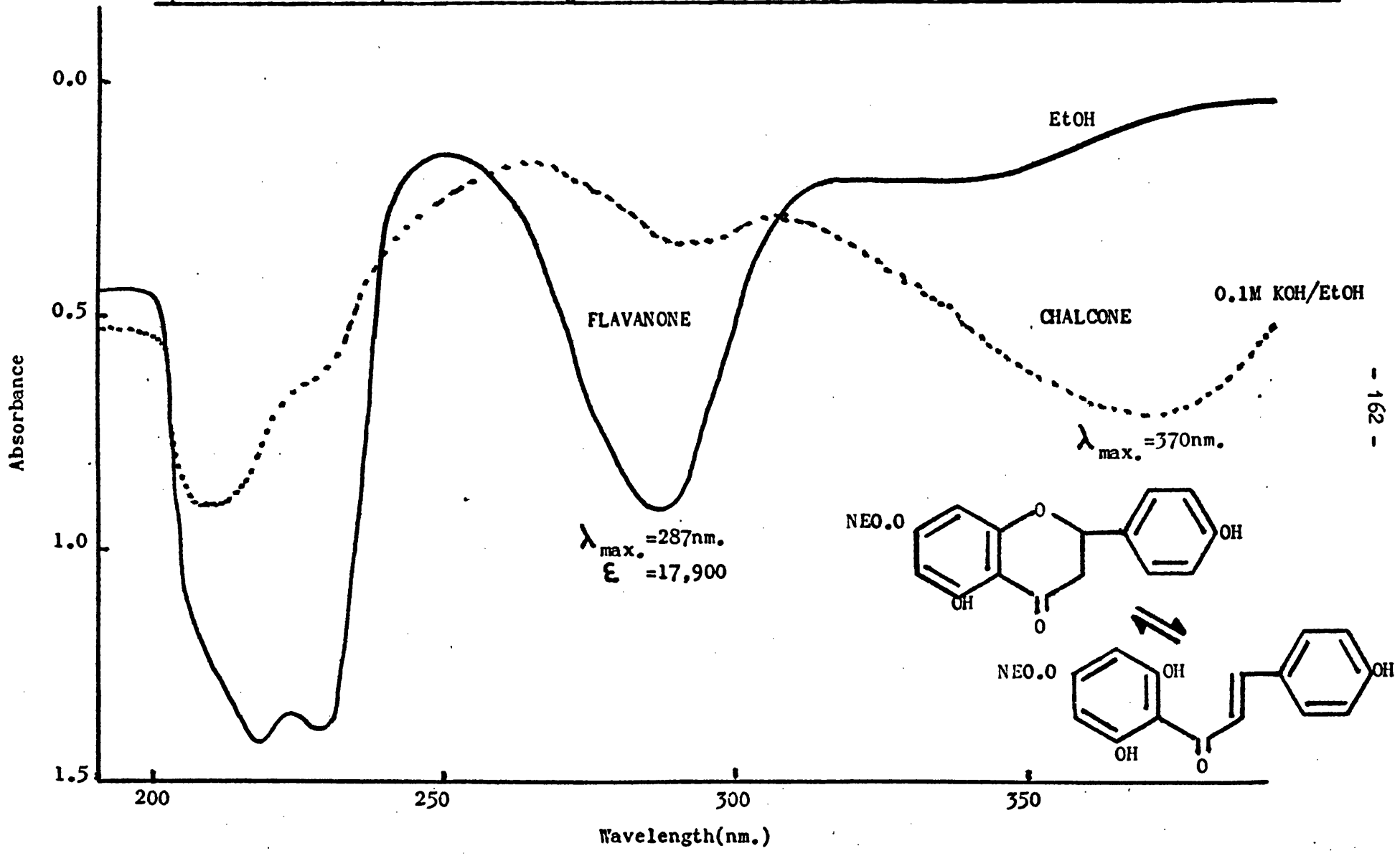
Spectrum 6 The PMR Spectrum of Phloroacetophenone-4'-β-neohesperidoside.(84).



Spectrum 7 The UV Spectrum of Phloroacetophenone-4-β-neohesperidoside in neutral or alkaline ethanol



Spectrum[8] The UV Spectrum of Naringin flavanone and Naringin chalcone in neutral or alkaline ethanol.



PART E

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